ISSN 0778-5569

OFFICIAL ORGAN
OF THE

EUROPEAN GROUP
OF LYMPHOLOGY
LATIN-MEDITERRANEAN
CHAPTER OF ISL
SOCIETÀ ITALIANA
DI LINFANGIOLOGIA

THE EUROPEAN JOURNAL OF lymphology and related problems

VOLUME 15 • Sp Co No. 43 • 2005

INDEXED IN EXCERPTA MEDICA

FIRST INTERNATIONAL SYMPOSIUM ON LYMPHATIC MICROCIRCULATION AND NEOPLASTIC METASTASIS

Parma - Italy

9-10 June 2005

THE EUROPEAN JOURNAL OF lymphology and related problems
VOLUME 15 - Sp Co No. 43 - 2005
INDEXED IN EXCERPTA MEDICA

GASPARE ASELLI AND THE DISCOVERY OF CHILIFEROUS VESSELS

A summary of the historical-medical report by BRUNO ZANOBIO

On the path traced by Andreas Vesal (1514-1564) the 16th Century marked the triumph, partly thanks to Italian scholars, of "new anatomy", which was, however, somewhat "static", being based essentially on the dissection of corpses.
The 17th Century saw the beginning of anatomic experimenting as the organism began to be considered as a machine that functions according to precise mathematic laws and mechanisms; this led to the birth of the "animated anatomy" and vivisection.
Characteristic of this century was the tendency of scholars to form scientific societies for the sake of conducting more fruitful experimental researches.
In July 1622 a group of five Milanese doctors, led undoubtedly, by this associative spirit, asked Gaspare Aselli (Cremona 1581 ca.-Milan 1625) to participate in their vivisection experiments.
Aselli, who had completed his medical studies in Pavia and worked as proto-surgeon for the Spanish army in Italy (a position he held until his appointment in 1624 as professor of anatomy at the University of Pavia), was already well known for his expertise in conducting anatomic-experimental researches.
In Milan. On July 23, 1622, therefore, in the presence of the abovement doctors, he proceeded to vivisect a dog in order to show them the recurrent nerves. After this demonstration he opened the abdominal cavity to show also the movement of the diaphragm. As he pushed down the intestinal mass and the stomach there suddenly appeared numerous tiny white threads that proceeded from the mesentery and intestines, and which he had never seen before. Once he was sure that these were not nerves, after careful thought he took a suitable scalpel and cut through one of the threads, which immediately gave out a white, milky liquid; in front of the astonished doctors the unexpected sight caused Asellio to shout out using the famous exclamation "Eureka!".
The dog, however, died immediately, and Aselli was unable to continue his experiment.
The next day Aselli repeated the experiment on another dog, but was disappointed to find no trace of the tiny vessels he had seen the day before. After careful consideration he remembered that, while the first dog had been well fed, the second was thin and underfed. This, then, was the explanation, which Aselli was to confirm in subsequent experiments on other animals.
The manuscript describing the discovery of cheliferous vessels is kept in the History Museum of the University of Pavia, while the printed book entitled *De Lactibus sive Lacteis venis Quarto Vasorum Mesaraicorum genera Novo Invento Gasperis Aselli* appeared posthumously in 1627, thanks to the good offices of Alessandro Tadino and Senator Settala, two of the doctors who had witnessed the discovery of the *fourth* type of vessels-the meseraic cheliferous vessels, joining the three known types (arteries, veins and nerves, which, as we know, were in those days believed to have vascular structure). The book was illustrated with plated that were created with the techinique of polychrome wood-engraving, used here for the first and last time and consequently earning it a special place in the history of printing.
In the description of his finds Aselli makes a number of errors that will be explained, from a critical- historical point of view, in the lecture, which will examine them in relation to the anatomical- physiological knowledge of the period. The demonstration of blood circulation, in fact, was only published later (William Harvey, 1628).
The discovery of Aselli encountered various difficulties before it was fully recognised, being firmly supported by some and strongly opposed by others. The first demonstration of cheliferous vessels in humans was performed several years later in 1654. A number of reports of great historical-medical interst were written on the distinction between cheliferous and lymphatic vessels (e.g. Thomas Bartholin, Olof Rudbeck Senior); of the 19th Century Italians, moreover, mention must be made especially of Paolo Mascagni and Bartolomeo Panizza.

GASPARE ASELLI E LA SCOPERTA DEI VASI CHILIFERI

Sunto della relazione storico-medico di BRUNO ZANOBIO

Sulla strada maestra tracciata da Andreas Vesal (1514-1564) il Cinquecento vede il trionfo, pure ad opera di studiosi italiani, della "nuova anatomia", peraltro piuttosto "statica", essendo fondata essenzialmente sulla dissezione del cadavere.
Nel Seicento si passa all'esperimento anatomico, venendo considerato l'organismo come macchina che funziona secondo precise leggi matematiche e meccaniche: da qui la nascita della "anatomia animata" comportante la vivisezione degli animali.
Caratteristica seicentesca è la tendenza degli studiosi a riunirsi in società scientifiche, onde potere più fruttuosamente condurre le ricerche sperimentali.
Certamente animati da spirito associativo, nel luglio del 1622, cinque medici milanesi chiedono a Gaspare Aselli (Cremona 1581 ca.-Milano 1625) di assistere ai suoi esperimenti di vivisezione.
Aselli compiuti gli studi medici a Pavia, protochirurgo degli eserciti spegnoli in Italia (carica che conservò sino alla nomina nel 1624 professore di anatomia all'università di Pavia), era già noto allora per la sua competenza nella conduzione di ricerche anatomo-sperimentali.
A Milano il 23 luglio 1622, aderendo alla richiesta dei medici suddetti, procedeva alla loro presenza alla vivisezione di un

cane per dimostrare i nervi ricorrenti. Terminata tale dimostrazione procedeva all'apertura della cavità addominale per dimostrare anche i movimenti del diaframma. Abbassata con la mano la massa addominale, assieme allo stomaco, gli apparvero allora, improvvisamente, numerosi sottili bianchissimi cordoncini decorrenti nel mesentere e negli intestini, e che mai gli era capitato di osservare. Accertato che non erano nervi, dopo meditata riflessione, con un adatto scalpello incideva uno di quei cordoncini, dal quale subito uscì un liquido bianco, simile al latte, il quale inaspettato spettacolo, lo spinse ad esclamare ai presenti il fatidico greco "Eureka!". Ma il cane subito morì, cosicché Aselli fu costretto a rinunciare a proeseguire il suo esperimento.
Nel giorno successivo Aselli ripetè l'esperimento su un altro cane, ma con delusione, non ritrovò i vasellini visti il giorno precedente. Dopo attenta valutazione del fatto, si ricordò che mentre il primo cane era ben pasciuto e sazio, il secondo era magro e digiuno: qui stava la spiegazione, confermata dalle successive sperimentazioni estese anche ad altri animali.
Il manoscritto concernente la scoperta dei vasi chiliferi è conservato nel Museo per la storia dell'Università di Pavia, mentre l'opera a stampa *De Lactibus sive Lacteis venis Quarto Vasorum Meseraicorum genera Novo Invento Gasparis Aselli*...comparve postuma nel 1627, grazie all'interessamento di Alessandro Tadino e Senatore Settala, due dei medici che avevano assistito alla scoperta del *quarto* genere di vasi, i vasi chiliferi meseraici, che si aggiungeva ai tre generi di vasi già noti (arterie, vene e nervi, ai quali, come noto, si attribuiva allora struttura vascolare). L'opera venne illustrata con tavole realizzate con la tecnica della xilografia policroma, usata per la prima ed ultima volta, tanto da occupare un posto particolare nella storia della tipografia.
Nella descrizione dei suoi reperti Aselli incorse in alcuni *errori* che verranno spiegati, sotto il profili critico-storico, nella relazione orale, che li esaminerà in relazione alle conoscenze anatomo-fisiologiche del tempo. La dimostrazione della circolazione del sangue, infatti, venne pubblicata solo successivamente (William Harvey, 1628).
La scoperta dell'Aselli incontrò pieno riconoscimento solo dopo varie difficoltà, essendovi stati fautori ed oppositori di essa. La prima dimostrazione dei vasi chiliferi nell'uomo venne effettuata alcuni anni dopo e precisamente nel 1654.
Notevole interesse storico-medico presentano i rapporti concernenti la distinzione fra vasi chiliferi e vasi linfatici (Thomas Bartholin, Olof Rudbeck senior). Circa questi ultimi merita ricordo, fra gli italiani ottocenteschi, Bartolomeo Panizza.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

ABSORBING LYMPHATIC VESSEL: LEUKOCYTE AND NEOPLASTIC CELL TRANSENDOTHELIAL MIGRATION MECHANISMS

Giacomo Azzali – Emeritus Professor
Director of the Laboratory of Lymphatology – Section of Human Anatomy, University of Parma
giacomo.azzali@unipr.it

Lymphatic microcirculation is prevalently composed by the Apparatus Lymphaticus Periphericus Absorbens (ALPA), which plays a basic role in preserving tissue homeostasis, in directing 50% of fluids and plasma proteins back to the bloodstream, in the immune response and represents the main pathway for the metastatic spread of tumour cells.
Firstly, we consider the organization and fine structure of lymphatic canalization in order to define the morphofunctional aspects of lymphatic vascular system, which is conveniently divided in two sectors: 1) lymphatic vessels with high and medium absorbent capacity (ALPA vessels and first tract of precollectors); 2) lymphatic vessels with prevalent flowing and conduction function (prelymph nodal and postlymph nodal collectors, main trunks and thoracic duct).
In the second part, we investigate the mechanisms regulating the transmigration of fluids, micro- and macromolecules and cells from the extravascular matrix into the lumen of lymphatic vessel. In this connection, the formation of intraendothelial channels by the ALPA vessel wall, in an interendothelial contacts-independent way, is explained. These channels are dynamic and unpermanent elements, whose numerical density changes during physiologic (dehydration) and pathologic (lymphedema) metabolic conditions. For a comparison with the lymphatic vessel, we consider models employed for blood vessels studying the role of adhesion molecules in leukocytes extravasation through high endothelial venules (HEV) of aggregate lymph nodules. Furthermore, the mechanisms of lymphocyte transendothelial migration from the interstitial matrix into the lumen of ALPA vessel, which occurs for the peculiar plasticity of endothelial lymphatic cells, are studied by means of ultrastructural serial sections and three-dimensional reconstructions.
In the third part, we evaluate the distribution and characterization of ALPA vessels (using transmission electron microscopy and D2-40 and LYVE-1 endothelial markers) in the tumour mass of some experimental tumours [(such as T84 adenocarcinoma, B16 melanoma and transgenic mice prostate adenocarcinoma (TRAMP)]. Besides, we analyse our original findings concerning the mechanisms which provide for the diffusion and transendothelial migration of tumour cells into the lumen of ALPA lymphatic vessels. In T84 cell line-inoculated mice, lymphatic vessels are only detectable in the peritumoural connective tissue. They show a monolayer endothelium lacking a continuous basal membrane and interendothelial gaps. Tumour cells with an invasive phenotype migrate in cords from the extravascular matrix of peritumoural connective tissue towards the lymphatic vessel; once arrived on the abluminal surface of the vessel, they take firm adhesion with the abluminal endothelial wall. Afterwards, ultrastructural and three-dimensional aspects show tumour cell passed into the lumen of the lymphatic vessel through an intraendothelial channel similar to that described by Azzali (1989, 2003) in human and other mammalians ALPA lymphatic vessels.
In B16 melanoma-inoculated nude mice, the subcutaneous tumour mass and gut metastases display lymphatic vessels only in the peripheral portion. Tumour cells with invasive phenotype, distributed in a diffused way within the extracellular matrix, after a firm adhesion to the abluminal endothelial wall migrate inside the ALPA lymphatic vessel in the same way of cells found in T84 adenocarcinoma-inoculated mice. These vessels do not present any ultrastructural degradation of the endothelial wall, nor modifications of interendothelial junctions.
In TRAMP transgenic mice, the prostate tumour mass, as the metastases in seminal vesicles and liver, show mostly collapsed ALPA lymphatic vessels in the middle and peripheral portions. They are formed by a continuous endothelial wall with irregular profile, lacking ultrastructural degradations, with prevalent *interdigitating* and *overlapping* interendothelial contacts and rare *end to end* contacts. The endothelial cells of these vessels present a non-nuclear cytoplasm with clear or electrondense matrix. Cells with an invasive phenotype, positive for SV40 marker, are diffused in the extravascular matrix and migrate individually towards the ALPA vessel; once reached the vessel, after a early stage of adhesion to the endothelial cell, these cells transmigrate into the lumen of the lymphatic vessel by an canalicular formation formed independently of interendothelial junctions. The latter are intact, with the plasma membranes of cytoplasmic extensions between adjacent cells fixed by *tight* and *gap* junctions. These ultrastructural and three-dimensional aspects emphatize a transmigration mechanism similar to that found in lymphatic vessels within B16 melanoma and T84 adenocarcinoma.
With regard to cell transendothelial migration process, hypotheses are advanced on the roles of VEGF-C and VEGF-D growth factors and other members of this family in intraendothelial channels induction and organization, as well as of adhesion molecules responsible of tumour cell-endothelial cell interactions.
Interpretations are made concerning the following items: a) the role of ALPA lymphatic vessel endothelial cells in cell chemoattraction and transendothelial migration; b) tumour cell transmigration in existing lymphatic vessels, rather than in newly formed ones after VEGF growth factors overexpression; c) possible modifications in the structure and function of ALPA vessel endothelium in tumours, if compared with those present in normal tissues.

On the whole, our morphological, ultrastructural and three-dimensional findings show the spatial arrangement of lymphatic vessels with high absorbent capacity in lymphatic vascular system and the plastic properties of endothelial cells in forming intraendothelial canalicular formation, and bring elucidations also on the still unclear mechanisms regulating fluids, macromolecules and cells transport into ALPA lymphatic vessels. Future investigations shall provide concrete contributions on the delicate molecular mechanisms controlling the interactions between tumour cell and endothelial cell of ALPA lymphatic vessel, in order to allow prospects and therapeutic strategies for increasing the diffusion of fluids (lymphedema) and for limiting or preventing the metastatic spread of tumour cells.

VASO LINFATICO ASSORBENTE: MECCANISMI DI MIGRAZIONE TRANSENDOTELIALE DEL LEUCOCITA E DELLA CELLULA TUMORALE

Il microcircolo linfatico è prevalentemente costituito dall'apparato linfatico periferico assorbente (ALPA) il quale gioca un ruolo importante nel mantenere l'omeostasi tissutale, nel facilitare il ritorno al sangue del 50% dei fluidi e delle proteine plasmatiche, nelle funzioni di difesa dell'organismo e nel costituire la porta principale per la diffusione metastatica delle cellule tumorali.
Nella prima parte della lettura viene presa in considerazione la organizzazione e la fine struttura della canalizzazione linfatica al fine di definire gli aspetti morfofunzionali del sistema vascolare linfatico, il quale viene opportunamente distinto in due settori: 1) vasi linfatici ad alta e media capacità assorbente (vaso ALPA e tratto iniziale del vaso precollettore); 2) vasi linfatici a prevalente funzione di scorrimento e di conduzione (vasi collettori pre- e postlinfonodali, grossi tronchi e dotto toracico).
Nella seconda parte, particolare attenzione viene riservata ai meccanismi che provvedono alla trasmigrazione dalla matrice extravasale all'interno del lume del vaso linfatico dei fluidi, delle micro- e macromolecole e delle cellule. Al riguardo viene sottolineata l'organizzazione da parte della parete endoteliale del vaso ALPA di canalicoli intraendoteliali che si formano in modo del tutto indipendente dai contatti interendoteliali. Detti canalicoli sono elementi dinamici non permanenti la cui densità numerica varia durante stadi metabolici fisiologici (disidratazione) e patologici (linfedema). Vengono presi in considerazione, al fine di una comparazione con il vaso linfatico, modelli impiegati per il vaso sanguifero circa il ruolo delle molecole di adesione nella extravasazione dei leucociti a livello delle venule postcapillari (HEV) dei linfonoduli aggregati. Così pure, mediante fettine seriate ultrastrutturali e la loro ricostruzione tridimensionale, sono stati studiati i meccanismi della migrazione transendoteliale del linfocito dalla matrice interstiziale all'interno del vaso linfatico ALPA che si realizza in virtù della peculiare plasticità della cellula endoteliale linfatica.
Nella terza parte viene presa in considerazione la distribuzione e la identificazione dei vasi linfatici ALPA (microscopio elettronico a trasmissione ed i markers D2-40 e LYVE-1) nella massa tumorale di tumori sperimentali [adenocarcinoma T84, melanoma B16 e adenocarcinoma della prostata di topi transgenici (TRAMP)]. Inoltre sono valutati gli originali referti circa i meccanismi che intervengono nella diffusione e nella migrazione transendoteliale della cellula tumorale all'interno del lume del vaso ALPA. Nei topi inoculati con la linea cellulare T84 i vasi linfatici risultano rilevabili solamente a livello del tessuto connettivo peritumorale. Essi si presentano caratterizzati da un endotelio monostratificato privo di membrana basale continua e di aperture interendoteliali. Le cellule tumorali con fenotipo invasivo migrano sotto forma di un agglomerato cordoniforme dalla matrice extravasale del connettivo peritumorale verso il vaso linfatico, e una volta raggiunta la superficie abluminale del vaso contraggono stretta adesione con la parete endoteliale abluminale. Dopo di chè, i quadri ultrastrutturali e tridimensionali mostrano la cellula tumorale trasmigrata nel lume del vaso linfatico attraverso una formazione canalicolare simile a quella descritta nei vasi linfatici ALPA dell'uomo e di numerosi mammiferi da Azzali (1989, 2003).
Nei topini nudi inoculati con melanoma B16 la massa tumorale sottocutanea e quella delle metastasi intestinali, lascia rilevare presenza di vasi linfatici solo nella sua porzione più periferica. Le cellule tumorali trasformate nel fenotipo invasivo distribuite in modo diffuso nella matrice extracellulare, dopo stretta adesione alla parete abluminale endoteliale migrano all'interno del vaso linfatico ALPA seguendo le stesse modalità rilevate nei topini inoculati con T84. Detti vasi non presentano segni di degradazione ultrastrutturale della parete endoteliale e così pure modificazioni delle giunzioni interendoteliali.
Nei topini transgenici TRAMP la massa tumorale della prostata, come quella delle metastasi a livello delle vescichette seminali e del fegato, presenta nella porzione media e periferica vasi linfatici ALPA per lo più collabiti. Essi sono costituiti da una parete endoteliale continua a profilo irregolare del tutto priva di degradazioni ultrastrutturali con contatti interendoteliali per lo più di tipo *interdigitating* e *overlapping*, mentre rare sono le *end to end*. Le cellule endoteliali di detti vasi sono caratterizzate da citoplasma non nucleare provvisto di matrice chiara o elettrondensa. Le cellule con fenotipo invasivo, positive al marker SV40, sono distribuite in modo diffuso nella matrice extravasale e migrano in modo individuale verso il vaso linfatico ALPA, raggiunto il quale, dopo una prima fase di adesione con la cellula endoteliale, trasmigrano nel lume del vaso linfatico attraverso una formazione canalicolare che si forma in modo del tutto indipendente dalle giunzioni interendoteliali. Queste ultime si presentano immodificate con le membrane delle espansioni citoplasmatiche di cellule endoteliali adiacenti fissate in modo costante da *tight* e *gap junctions*. Detti quadri ultrastrutturali e tridimensionali ottenuti sottolineano un meccanismo transmigratorio molto simile a quello rilevato nei vasi linfatici del melanoma B16 e dell'adenocarcinoma T84.
Riguardo al processo migratorio transendoteliale delle cellule, sono prospettate supposizioni sul compito che possono svolgere i fattori di crescita VEGF-C e VEGF-D e altri membri di questa vasta famiglia circa la induzione e la organizzazione delle formazioni canalicolari, e così pure quello delle mole-

cole di adesione responsabili delle interazioni "cellula tumorale-cellula endoteliale".
Vengono formulate interpretazioni circa: a) il ruolo svolto dalle cellule endoteliali del vaso ALPA riguardo la chemioattrazione e la migrazione transendoteliale delle cellule; b) la transmigrazione delle cellule cancerogene nei vasi linfatici già esistenti piuttosto che in nuovi vasi linfatici generati dalla sovraespressione dei fattori di crescita VEGF; c) le eventuali modificazioni della struttura e della funzione dell'endotelio linfatico nei vasi ALPA dei tumori rispetto a quella dei vasi dei tessuti normali.
Nel complesso i reperti morfologici, ultrastrutturali e tridimensionali da noi illustrati consentono di sottolineare l'individuazione spaziale nel sistema vascolare linfatico del vaso linfatico ad alta capacità assorbente e le proprietà plastiche della cellula endoteliale nell'organizzare formazioni canalicolari intraendoteliali, portando anche un contributo chiarificatore sui meccanismi, ancora non del tutto chiari, che presiedono al trasporto all'interno del vaso linfatico ALPA dei fluidi, delle macromolecole ed in particolar modo delle cellule. Compito di future ricerche il fornire concreti contributi sui delicati meccanismi molecolari che presiedono alle interazioni cellula tumorale-cellula endoteliale del vaso linfatico ALPA al fine di consentire prospettive e strategie terapeutiche per incrementare la diffusione di liquidi (linfedema) e per limitare o impedire la disseminazione metastatica delle cellule cancerogene.

REFERENCES

Azzali G, Orlandini G, Bucci G. (1989) Morphological characters of. the absorbing peripheral lymphatic vessel by TEM, SEM and three-dimensional models. Prog Clin Biol Res.; 295:487-92.
Azzali G. (2003) Transendothelial transport and migration in vessels of the apparatus lymphaticus periphericus absorbens (ALPA), Int Rev Cytol.;230:41-87.
Azzali G. (2003) Structure, lymphatic vascularization and lymphocyte migration in mucosa-associated lymphoid tissue, Immunol Rev.;195:178-89.

THE EUROPEAN JOURNAL OF
lymphology
and related problems
VOLUME 15 • Sp Co.No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

MOLECULAR PATHWAYS FOR LYMPHANGIOGENESIS AND THEIR ROLE IN HUMAN DISEASE

Steven A. Stacker1, Carol Caesar1, Rachael Inder1, Megan Baldwin1, Brad McColl1, Sally Roufail1, Richard Williams2, Tony Hughes3, Kari Alitalo4 & Marc G. Achen1.

[1]Angiogenesis Laboratory, Ludwig Institute for Cancer Research, PO Box 2008, Royal Melbourne Hospital, 3050, Victoria, AUSTRALIA, [2]Department of Pathology, Faculty of Medicine, Dentistry and Health Sciences and [3]Department of Pharmacology, University of Melbourne, Victoria, Australia, [4]Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Biomedicum Helsinki and Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland. E-mail Steven.stacker@ludwig.edu.au

The dysfunction or proliferation of lymphatic vessels (lymphangiogenesis) is linked to a number of pathological conditions including lymphedema and cancer (Baldwin et al., 2002; Stacker et al., 2002). The recent discovery and characterisation of the lymphangiogenic growth factors vascular endothelial growth factor-C (VEGF-C) and VEGF-D and of their receptor on lymphatic endothelial cells, VEGFR-3, has provided an understanding of the molecular mechanisms controlling the growth of lymphatic vessels (for review see Stacker et al., 2004a). In addition, other genes and protein markers have been identified with specificity for lymphatic endothelium that have enhanced the characterization and isolation of lymphatic endothelial cells.

In cancer there is evidence that expression of the lymphangiogenic factors VEGF-C and VEGF-D can induce the formation of tumor lymphatic vessels which are associated with spread of the primary tumor to local lymph nodes (Mandriota et al., 2001; Skobe et al., 2001; Stacker et al., 2001). Human tumor xenografts engineered to express these factors have increased lymphatic vessels and a greater capacity to produce lymphogenous metastasis. In these studies the use of monoclonal antibodies and other inhibitors to the factors can prevent these effects (He et al., 2002; Stacker et al., 2001). These studies using animal models are supported by the correlation found between the expression of these factors in primary human tumors and clinical parameters relating to tumor metastasis, in particular lymph node spread (Stacker et al., 2002; Stacker et al., 2004b). Hence, it may be beneficial to inhibit these molecules in cancer to restrict metastatic spread (Achen et al., 2005).

Conversely, the use of lymphangiogenic growth factors to induce the formation of new lymphatic vessels provides a potential method to treat conditions such as lymphedema where lymphatic vessel function is inadequate (Baldwin et al., 2002). The pro-lymphangiogenic effects could also be of benefit in areas such as tissue repair. The proof-of-principle for using lymphangiogenic growth factors to grow lymphatic vessels in healthy tissue and tissue affected by lymphedema has been described in the literature (Rutanen et al., 2004; Szuba et al., 2002).

Our growing understanding of the molecules that control lymphangiogenesis allows us to design more specific drugs with which to manipulate the relevant signalling pathways (Achen et al., 2005; Stacker et al., 2004a). Modulating these pathways and other molecules with specificity to the lymphatic system could offer alternative treatments for a number of important clinical conditions.

REFERENCES

Achen,M.G., McColl,B.K., and Stacker,S.A. (2005). Focus on lymphangiogenesis in tumor metastasis. Cancer Cell *7*, 121-127.
Baldwin,M.E., Stacker,S.A., and Achen,M.G. (2002). Molecular control of lymphangiogenesis. Bioessays *24*, 1030-1040.
He,Y., Kozaki,K., Karpanen,T., Koshikawa,K., Yla-Herttuala,S., Takahashi,T., and Alitalo,K. (2002). Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. J Natl Cancer Inst. *94*, 819-825.
Mandriota,S.J., Jussila,L., Jeltsch,M., Compagni,A., Baetens,D., Prevo,R., Banerji,S., Huarte,J., Montesano,R., Jackson,D.G., Orci,L., Alitalo,K., Christofori,G., and Pepper,M.S. (2001). Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. EMBO J. *20*, 672-682.
Rutanen,J., Rissanen,T.T., Markkanen,J.E., Gruchala,M., Silvennoinen,P., Kivela,A., Hedman,A., Hedman,M., Heikura,T., Orden,M.R., Stacker,S.A., Achen,M.G., Hartikainen,J., and Yla-Herttuala,S. (2004). Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart. Circulation *109*, 1029-1035.
Skobe,M., Hawighorst,T., Jackson,D.G., Prevo,R., Janes,L., Velasco,P., Riccardi,L., Alitalo,K., Claffey,K., and Detmar,M. (2001). Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat. Med. *7*, 192-198.
Stacker,S.A., Achen,M.G., Jussila,L., Baldwin,M.E., and Alitalo,K. (2002). Lymphangiogenesis and cancer metastasis. Nat. Rev. Cancer *2*, 573-583.
Stacker,S.A., Caesar,C., Baldwin,M.E., Thornton,G.E., Williams,R.A., Prevo,R., Jackson,D.G., Nishikawa,S.-I., Kubo,H., and Achen,M.G. (2001). VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat. Med. *7*, 186-191.
Stacker,S.A., Hughes,R.A., and Achen,M.G. (2004a). Molecular targeting of lymphatics for therapy. Current Pharmaceutical Design *10*, 65-74.
Stacker,S.A., Williams,R.A., and Achen,M.G. (2004b). Lymphangiogenic growth factors as markers of tumor metastasis. APMIS *112*, 539-549.
Szuba,A., Skobe,M., Karkkainen,M.J., Shin,W.S., Beynet,D.P., Rockson,N.B., Dakhil,N., Spilman,S., Goris,M.L., Strauss,H.W., Quertermous,T., Alitalo,K., and Rockson,S.G. (2002). Therapeutic lymphangiogenesis with human recombinant VEGF-C. FASEB. J. *16*, 1985-1987.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

NUCLEAR MEDICINE IN THE STUDY OF LYMPHATIC DISORDERS

Bourgeois Pierre, MD, PhD,
Université Libre de Bruxelles, Institute Jules Bordet, Service of Nuclear Medicine, Brussels, Belgium
E-mail: Pierre.bourgeois@bordet.be

Nuclear medicine techniques allowing the study of the lymphatic system diseases include:
- the lymphoscintigraphic investigations (LySc) using 99mTc-labeled colloids,
- the investigations with Positron Emission Tomography (PET) after administration of various tracers.

In the past, the lymphoscintigraphic investigations using 99mTc-labeled colloids were used to demonstrate the metastatic invasion of several lymph nodes groups (with prognostic implications as for instance in breast cancer with the lymphoscintigraphic investigations of the internal mammary nodes) and/or to define the spatial localisation of lymph nodes to be irradiated (the internal mammary nodes in breast cancer). Actually, they are used in the management of the oedematous situations, primary or secondary, and have largely proven their interest in these indications. Now, they are also and more and more used to demonstrate in vivo the lymph node(s) draining one tumour bearing area and at risk to be invaded by metastatic cells (the so-called "sentinel lymph node-s"). Today, quite all types of cancer are concerned by this application: cutaneous tumours as melanomas or Merkel's ones, mammary tumours, head and neck ones, prostatic tumours.
If LySc allow to study directly the lymphatic vessels and lymph nodes, the PET techniques after intravenous administration of various tracers on the another hand allow to demonstrate the metabolic activity of primary lymph node diseases (lymphoma) and/or the presence in the lymph nodes of metabolically active secundary metastatic disease.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005

RADIATION THERAPY IN DOGS WITH SPONTANEOUS TUMORS AND ITS IMPACT ON PLASMA VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION

1 Wergin M, Olerth S, Rohrer C, Allemann K, Laluhovà D, Kaser-Hotz B
1Diagnostic Imaging and Radio-Oncology, Vetsuisse Faculty University of Zürich, Switzerland
email: mwergin@vetclinics.unizh.ch

Cancer is the number one cause of death in dogs. The overall cancer incidence rates per 100 000 individuals per year demonstrated at risk, is 381, which is comparable with humans. In the last few decades, many clinical trials and retrospective studies could show the efficacy of surgery, radiation therapy and chemotherapy in the treatment of cancer in veterinary medicine. This knowledge brought cancer therapy in to the focus of many veterinarians and owners. In particular, radiation therapy (RT) is an effective treatment modality, just as it is for human cancer patients. In radiation therapy two main treatment options are given. First, a treatment with the intent to cure the canine patient. Curative radiation includes 12 to 17 fractions with 3 to 4 Gy per fraction. Secondary, a palliative treatment might be chosen. The aim of the palliative treatment is to increase life quality. This treatment does not exceed the quality of life of the patient. A palliative protocol is given over 10-14 days, including 4 to 5 fractions with 6 Gy. The goal of radiation therapy is to destroy the reproductive capacity of the tumor tissue without excessive damage to surrounding normal tissues. The probability of tissue damage is dependent upon the total applied dose, as well as on fraction size. In order to allow for repair in normal tissues, the total dose is divided into a series of small fractions which are administered over a period of time. These time-dose-fraction relationships are very critical for the success of a radiation treatment, and can also vary dependent upon the type of tumor. Ionizing radiation kills cells by the deposition of energy (e.g. electrons or photons) on or near the DNA. The DNA damage is fixed if oxygen is present. The DNA damage then leads to cell death.

Unfortunately, the efficacy of radiation treatment is still limited. Variations in radiosensitvity of certain tumors is a major problem in treatment planning and prognosis. Radiosensitivity of tumor cell lines was tested *in vitro*, or *in vivo*. Interestingly, radiosensitivity *in vitro* did not always correlate to radiosensitivity *in vivo* (Taghian A et al, 1993). This finding was the basis of the assumption that radioresponsiveness is not only dependent on tumor cells, but also on other cells in the tumor, e.g. tumor endothelial cells. It is believed that a crosstalk between tumor and endothelial cells has an impact on the radiosensitivity of solid tumors. In this context, the vascular endothelial growth factor (VEGF) play an important role. VEGF is one of the major pro-angiogenic factors acting preferentially on endothelial cells. VEGF acts through the binding to its receptors KDR1 and Flt-1. These receptors are expressed on the endothelial cell surface as well as on some tumor cells. VEGF promotes tumor angiogenesis, endothelial cell survival and vessel maintenance of immature vessels. VEGF also has a protective effect on tumor cells expressing VEGF receptors. VEGF is involved in development and growth of a wide variety of different tumors. VEGF is actively secreted from tumor cells and its soluble form ($VEGF_{165}$) is detectable in the blood compartment. High plasma VEGF was shown to be a significant predictor of reduced overall survival and of earlier onset of local recurrence in human patients. Therefore, high serum and plasma VEGF concentrations are a clinically useful negative prognostic indicator.

The upregulation of the VEGF expression and release from tumor cells can be activated by several factors. First, under hypoxic conditions, the hypoxia inducible factor-1 (HIF-1) is stabilized. HIF-1 can bind to DNA at specific regions, called hypoxia responsive elements (HRES). This element is sufficient to mediate upregulation of the transcription of VEGF. This upregulation leads to increased angiogenesis, to overcome hypoxia. Secondary, ionizing radiation can lead to phosphorylation of the PI-3kinase via the epidermal growth factor receptor (EGFR) in tumor cells. The phophorylation of the EGFR activates the ERK pathway, a mitogen activated protein kinase (MAPK) pathway. This activation is mediates a clear upregulation of VEGF expression in tumor cells. This increased expression is dose dependent. Cells exposed to low doses of radiation (1 Gy) caused prolonged activation of the ERK pathway. In contrast, higher doses of radiation (6 Gy) caused a much weaker activation. In summary, VEGF might be upregulated during fractionated radiation therapy via activation of the ERK pathway in a dose dependent manner, and this effect can be counterbalanced by reoxygenation, via destabilization of HIF-1, during RT.

The upregulation of VEGF by ionizing radiation itself could result in a self limiting effect of RT. To study this hypothesis VEGF was measured in the plasma of dogs with spontaneous tumors. Dogs in this study were irradiated either with a curative or a palliative intent. VEGF concentration was determined by using a commercially available human VEGF enzyme linked immunosorbent assay (ELISA; R&D Systems Inc., Abingdon, United Kingdom). In the palliative group (5x6 Gy/ 4x6 Gy), blood samples were taken before and after each fraction, while from curatively treated dogs (12-16x3.5 Gy or 15-17x3 Gy) blood samples were taken before and after fraction 1, 3, 6, 8, 10, 12, 14 and 16.

In the first part of this study, we were interested if dogs with different tumor types would differ in their baseline plasma

VEGF. Low levels of plasma VEGF were found in dogs with epulis, a benign tumour with a minimal metastatic potential. In contrast, high plasma VEGF levels were found in more aggressive tumours with a high metastatic potential, such as oral melanoma, carcinoma and osteosarcoma. These data indicate a close relationship between plasma VEGF and aggressiveness of the tumor. Secondarily, the effect of fractionated RT on VEGF levels in the plasma of tumor bearing dogs was analyzed and a tendency for increased plasma VEGF level in curatively treated dogs was found. These results are in correspondence with the in vitro study, where a low radiation dose resulted in pronounced activation of the VEGF expression. VEGF concentrations immediately before and after dose application did not differ.

In human medicine, an association between high serum or plasma VEGF level and a shortened survival time was seen. Interestingly, high preirradiation plasma VEGF levels in dogs with spontaneous tumors also resulted in a shortened survival time. This was seen in curatively and palliatively treated dogs. This prediction was not dependent on tumor histology or tumor stage. Additionally, we found lowest mean plasma VEGF levels in dogs with a complete response to therapy in both treatment groups. Correspondingly, palliatively treated dogs with a low plasma VEGF level that responded to therapy also had a significantly longer time to treatment failure and a longer life span.

In conclusion, a close relationship between plasma VEGF and the aggressiveness of the tumor was proven and plasma VEGF is a good prognostic indicator for dog patients receiving radiation therapy.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 - Sp Co No. 43 - 2005
INDEXED IN EXCERPTA MEDICA

IMAGING IN LYMPHATIC DISEASES

S. Michelini, A. Failla, G. Moneta, G. Paroni Sterbini, L. Mariani
San Giovanni Battista Hospital – Rome – Italy
sandro.michelini@fastwebnet.it

Today the lymphatic diseases are studied with the clinical exam but, above all, by means the instrumental investigations.
Lymphoscintigraphy represents the diagnostic gold standard in the study of lymphedema, both in primary kinds and in secondary one.
The exam provides morphological and functional indications on lymphatic circulation, but it's necessary a good interpretation of dates by sanitary operators.
The morphological aspects demonstrated by the lymphoscintigraphy can be :
- Hypo genesis or agenesis of lymphatic stations or ways one.
- The presence and the extension of dermal back flow.
- The presence of lymph nodal stops in the middle share of the limb (knee in the leg, elbow in the arm).
- The presence of alternative ways.

In our study on three hundred ninety two patients (one hundred fifty three males and two hundred twenty-nine females age ranging between twelve and seventy-four years primary, secondary, post traumatic, from disuse and functional – for example: after deep venous thrombosis) the dermal back flow generally was proportioned to the clinical stage of lymphedema; it was possible a partially regressinon after the treatment and, in some cases, appeared also with healthy lymphatic stations (for example in the post-phlebitic syndrome or in iatrogenic lesions of trunks).
Dermal back flow was present in the area of the trauma. In these cases, if the lymph nodes of the root of the limb was normal, the prognosis was good. On the contrary, in case of hypogenesis of lymph nodal station at root of the limb, the prognosis was bad.
In depending lymphedema was observed from disuse there was a bilateral dermal back flow (usually at lower limbs). In these cases usually the inguinal stations and latero-iliac ways were normal.
In the post phlebitic syndrome the dermal back flow was monolateral and rarely was associated with a lymphatic hypogenesis at the root of the limb. lymph nodal stops, generally at knee level in the lower limbs, at elbow level in the upper limbs, were observed.
The lymph nodes normally were not visible. Their presence was a sign of difficult of lymphatic down flow.
They were also present in the health limbs of women with mastectomies as a witness of predisposition to the lymph stasis.
The alternative ways, well represented in some cases, addressed the physical treatment to best drainage.
In conclusion the lymphoscintigraphy gives us important informations on diagnosis, on therapeutical approach and on prognosis in each patient.
Some aspects of the exam must be better focused in function of the clinical case for the necessary best therapy.
High resolution echography help us to confirm the localization of oedema, its extension, the prevailing presence of oedema in supra or sub-fascial tissues and the liquid and or fibro-sclerotic component.
The parameters to take into consideration during the exam are:
the supra and subfascial tissular thickness;
the echogenicity;
the compressibility;
the lymphnodes characteristics.
With the echographic exam we can observe:
a substantial iso-echogenicity (above all of the supra-fascial tissue in normal subjects) ;
a prevailing ipo-echogenicity corresponding to the fluid component (frame A);
a simultaneously presence of hypo-echogenic and hyper-echogenic zones of the tissues (frame B);
a prevailing hyper-echogenicity of the tissues corresponding to the fibrosis (frame C).

In normal subjects we observe in both of the two limbs a coinciding echogenicity and thickness of supra and sub-fascial tissue.
At various level of supra-fascial thickness we can see an interposition of hyper-ecogenic stripes corresponding to the fat tissue.
When we observe a prevailing and homogeneous cutaneous fascial hypo-echogenicity, we are in presence of water spraiding inside the tissues, sometimes with an interposition of lymphatic lakes and/or canals (frame A).
In these cases a compressibility of tissues determines a substantial reduction of supra-fascial thickness tissue.
In other cases we observe an un-omogeneous hypo-echogenicity of the supra-fascial thickness caused by presence of water with several hyper-echogenic stripes of zones due to fibrosis of tissue. It's possible in these cases to observe some lymphatic lakes and/or canals. The tissutal compressibility is possible but the decrease of supra-fascial thickness is less (frame B).
In other cases we observe more diffuse hyper-echogenic supra-fascial stripes and/or zones caused by tissutal fibrosis. In these cases there is a poor hypo-echogenic component. It's possible to observe also some lymphatic lakes or canals. Very poor, or absent, it is, in these cases, the tissutal compressibility (frame C).
We consider also interesting, in our experience, to analyze the morphological aspects of lymph-nodes at the root of the limbs suffering from lymphedema. We can observe an unsuccessful visualization of them; a diffuse or rare volumetric increase with a poor hypo-echogenic peripheric component (above all in post lymphangitis , post phlebitic or chronic leg ulcer cases); a diffuse or rare volumetric increase with a great hypo-echogenic peripheric component (above all in acute lymphangitic or phlebitic cases).
In our casuistry we observed 72 patients with echographic frame A, 65 with echographic frame B and 49 with echographic

frame C. In all patients we performed, for 2 intensive weeks a tailored physical tratment (respiratory gymnastic, M.L.D., sequential pressotherapy, under-bandages isotonic gymnastic).
In the first clinical stage we observed all normal echogenic frame.
In the second clinical stage 63 cases of frame A and 5 of frame B;in the third clinical stage 9 frame A, 49 frame B and 9 frame C; in the fourth clinical stage 11 frame B and 9 frame C; in the fifth clinical stage only 4 frame C. There was a substantial coincidence between the echographic frame and the clinical stage.
In all patients with echographic frame A we observed a remarkable decrease, after treatment, of supra-fascial tissutal thickness. In 31% of patients we saw a regression to normal frame.
In the patients with echographic frame B we observed, after treatment, a decrease of hypo-echogenic component (corresponding to the fluid component of oedema); in 24,5% a regression to the previous clinical stage.
In all cases the echographic frame was unvaried after treatment.
In the patients with echographic frame C was observed a very low decrease of hypo-echogenic component of supra-fascial thickness.
In 24,7% it is visible a regression to the previous clinical stage.
In all cases an unvaried echographic frame was observed. There are the echographic frame modifications in function of the treatments.
The echographic frame of lymphedema can help us also for the prognosis. In fact the echographic frame A can regress to the normal frame. The complete reversibility is not possible for the other echographic frames (B and C).
Under the clinical point of view the reversibility is completely possible for the frame A, partially for the frame B and very low for the echographic frame C.
In conclusion we can asses that the high resolution echography allows us to confirm the diagnosis of lymphedema, the monitoring of the results for the treatment and to provide also the prognostic indications.
Computering Tomography shows some important informations about the tissular caractheristics, both of the supra-fascial compartment and of the sub-fascial one.
The Author studied 220 patients suffering from lymphedema (86 with primary kind, 134 with secondary kind), age ranging between 12 and 83 years.
The study focused above all on the main signal present in supra-fascial compartiment of the limb at various level of it.
The values strongly negative (-70, -80) was corresponding to the fat tissue; the values ranging between –10 and +15 represented the main water component; The values over +50, +60 testified the presence of tissular perilymphangiosclerosis. To respect the quality-factor we accepted and examined only the images with 'standard-deviation' within 10.
The parameters examinated was :
modification of the thikness of supra and sub-fascial compartiment at various level of the limb;
the main tissular component of supra-fascial tissue at various level;
the assessment of the main articulations of the limb;
the trophism of various muscolar component of the limb.
In this way the computering tomography permit to address the therapeutical approach (general and particular) on all the anatomical component to treat with the proper rehabilitative protocol: CPT+drugs (when it was present the combined presence of water and perilymphangiosclerotic supra-fascial tissular component – 37% of subjects); CPT+drugs+microsurgery (when it was prevailing the idric supra-fascial tissular component- 47% of subjects); CPT+liposuction (in subject with prevailing suprafascial fat component - 16% of patients).
In conclusion the Computering Tomography permit to define the various diagnostic aspects of lymphedema and the choice of the tailored therapeutical protocol; the exam is important also to the monitoring of the illness.
Also the Magnetic Resonance, when indicated associated with the injection of the contrast liquid, can help us to define the ultrastructural features of the tissue, above all of the supra-fascial thickness.
The Lymphography, today, is indicated for the morphological study of some cases of chiloperitoneous, chilothorax or in tumoral secondary kind of lymph-stasis.
All these instrumental techniques favorise the diagnosis and permit us to define the clinical approach; each of them give us some important informations to realize the proper tailored rehabilitaive project in lymphedema patient.

REFERENCES

1. Badini A, Fulcheri E, Campisi C, Boccardo F. A new approach in histopathological diagnosis of lymphedema: pathophysiological and therapeutic implications. Lymphology 1996; 29 (S): 190-198.
2. Benda K., Lebloch D., Bendova M.: Prevention of primary lymphedema- Possible Lymphology 31 (Suppl) 1998: 465-468.
3. Campisi C, Jiménez Cossio JA, Pissas A, Leduc A, Michelini S, Boccardo F, Zilli A. Prevention of Secondary Lymphedema: Prospects for the Future. Lymphology 1998; 31 (Suppl): 513-515.
4. Insua EM: "Diagnostico por imagen de la patologìa venolinfàtica de las extremidades". Linfologìa, n.3, año 2, 15-20, 1996.
5. Matter D.,Grosshans E., Muller J., Furderer C., Mathelin C., Warter C., Bellocq J-B., Marllet C.. Apport de l'échographie à l'imagerie des vaisseaux lymphatiques par rapport aux autres méthodes. J. Radiol., 1991, Vol. 83, p. 599-609
6. Michelini S., Failla A., Paroni Sterbini G.L., Micci A., Santoro A., Valle G.: Limb phlebolimphedema: diagnostic non invasive approach and therapeutical implications. The European Journal of lymphology. Vol.5, n°20:103-8 1995
7. Michelini S., Failla A.: Linfedemi: Inquadramento diagnostico clinico e strumentale. Minerva Cardioangiologica. 1997. Vol°45, n°6:11-15.
8. Witte CL, Witte MH. Consensus and dogma. Lymphology 1998 Sep; 31(3): 98-100.
9. Case T.C., Witte C.L., Witte M.H.. Magnetic resonance imaging in human lymphedema: comparison with lymphangioscintigraphy. Magnetic Resonance Imaging 1992; 10:549-558
10. Dimakakos P.B., Stephanopoulos Th, Antoniades P., Antoniou A., Gouliamos A., Rizos, D. MRI and Ultrasonographic Findings in the Investigation of Lymphedema and Lipedema Int Surg 1997; 82:411-416

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2008
INDEXED IN EXCERPTA MEDICA

GENES, RECEPTORS, LYMPHANGIOTUMORIGENESIS, TUMOR LYMPHANGIOGENESIS, AND TRANSLATIONAL LYMPHOLOGY

Marlys H. Witte, Kimberly Jones, Michael J. Bernas, Charles L. Witte.
Department of Surgery, University of Arizona, USA

The lymphatic system of lymphatic vessels, lymph, lymph nodes and lymphocytes (and other immunocytes) comprises a distinctive vasculature (open junctions, anchoring filaments, valves, and intrinsic contractility) different from but similar to the blood vasculature; an integral component of the plasma-tissue fluid-lymph circulation (the "blood-lymph loop"); and the center of the immune system. Lymphatic vessels are involved in diverse pathologic processes both analogous to and distinct from, albeit far less studied and understood than, those affecting the blood vasculature. Interference with the blood-lymph loop produces swelling (an imbalance between lymph formation [regulated by Starling's law of transcapillary fluid exchange] and lymph absorption), scarring, nutritional and/or immunodysregulatory disorders as well as disturbances in lymph(hem)angiogenesis (the lymphedema-angiodysplasia [LE-AD] syndromes). The lymphatic system is also the stage on which key events during cancer development and progression are played out. Historically, lymph was first thought to be the tissue "gel" from which cancers arise and subsequently, until the present day, lymphatic system involvement has formed the basis for evaluation, prognostication, and/or both operative and non-operative treatment of most cancers.

Recently, advances in molecular biology and the unlocking of the human genome have ushered in the era of "molecular lymphology." These discoveries, new concepts, and techniques, framed by the pioneering studies of the forerunners and founders of the discipline of lymphology, are beginning to unravel the poorly understood embryonic development, physiology and pathophysiology of the lymphatic system. Intimately associated with tissue and cellular events, the implicated molecular inventory of lymphatic growth factor ligands (particularly the VEGF [C and D] and angiopoietin [1 and 2] families), endothelial receptors, transcription factors, and genes appear to modulate, by as yet unclear and possibly unrelated pathways, both "lymphvasculogenesis" and "lymphangiogenesis" (including also "tumor lymphangiogenesis" and "lymphangiotumorigenesis"). These processes in the lymphatic vasculature resemble but are distinct from those involving the blood vasculature (more properly termed "hemovasculogenesis" and "hemangiogenesis".)

Aside from the chromosomal aneuploidies commonly associated with lymphatic anomalies and even fetal demise, a "reverse genetics" approach has now identified specific genes for three monogenic LE-AD conditions, and loci have been mapped for several others. Furthermore, there are close to 40 distinct familial syndromes, most OMIM-listed or cross-referenced, affecting the lymphatic segment of the vasculature. Mutations have been identified in endothelial receptor VEGFR3 for lymphatic growth factor VEGF-C in a subpopulation of Milroy syndrome of lymphatic hypoplasia, winged helix transcription factor FOXC2 uniformly in hundreds of patients with lymphedema-distichiasis syndrome with a hyperplastic incompetent lymphatic system; and transcription factor SOX18 in 2 families with autosomal recessive hypotrichosis-lymphedema-telangiectasia syndrome. Through a "forward genetics" approach, transgenic mouse models of LE-AD have implicated still other growth factor ligand-receptor families (e.g., the angiopoeitin-tie system) and transcription factors in lymphatic growth and development.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 - Sp Co No. 43 - 2005
INDEXED IN EXCERPTA MEDICA

ISOLATION, CHARACTERIZATION AND EXPANSION OF HUMAN LYMPHATIC ENDOTHELIAL CELLS IN VITRO

Emirena Garrafa, Giulio Alessandri, Arnaldo Caruso*

Department of Microbiology, University of Brescia - Italy
** Neuroangiogenesis, National Institute "C. Besta" Milan - Italy*
emirenagarrafa@libero.it

DISCUSSION

Very few attempts have been made in the past to isolate Lymphatic endothelial cells (LECs), mainly using lymphatic from vascular tumors (Mancardi,1999, Weninger 1999). Due to the lack of specific markers, a stable phenotype in culture and the histogenetic origin of this expanded population could not be defined, but the recent identification of novel lymphatic markers such Podoplanin, a transmembrane mucoprotein (Matsui et al, 1999), hyaluronidase receptor LYVE-1 (Banerji et al, 1999), VEGFR-3 (Jussila et al 2002), transcription factor PROX-1 (Petrova et al, 2002) and D2-40, recognizing an O-linked sialoglycoprotein (Kahn et al, 2002) as made it possible. Furthermore the identification of specific lymphatic growth factors, like VEGF-C, and substrates recently has made possible the isolation and growth of LECs from derma by using two different markers: podoplanin and VEGFR-3 (Makinen, Kriehuber). Here we described for the first time the isolation of LECs from palatine tonsil and thoracic duct using a mAb recognizing the O-linked sialoglycoprptein D2-40 specifically expressed on LECs. PT as source of LECs are extremely usefull because is easy to obtain as discarded material of therapeuthic surgical procedures and because of their dimension, weight and richness in lymphatic capillaries. Generation of LEC cultures required tissue dispersion, removal of non adherent cells after 12-18 h of plating, immune preselection with UEA-1-coated magnetic beads followed by a second selection with magnetic beads coated with D2-40 mAb. It is known that LECs directly interact *in vivo* with the extracellular matrix (Gerli et al, 2000) and indeed both PT-LECs and TD-LECs were successfully propagated on collagen and fibronectin coated flasks in the presence of EGM added with VEGF-C. However, LECs cultured on collagen and fibronectin adhere better and proliferate more efficiently then LECs grown on culture dishes coated with collagen alone (Grinnet et al 1981, Kleinman et al 1981),according with data that fibronectin provides additional signal for adhesion, survival and proliferation of LECs. PT-LECs and TD-LECs were morphologically similar to BECs, although their shape appeared more elongated. Most of the known blood vascular markers, such as CD31, vWf and UEA-1, are present at lower levels or almost absent, like KDR, in agreement with previous studies performed with LECs derived from derma (Kriehuber et al., 2001). Also lymphatic markers are differentially represented. PT-LECs were strongly stained by D2-40 mAb, almost all express Prox-1 and only few element were LYVE-1$^+$ and Podoplanin$^+$ while almost all TD-LECs in cultures equally expressed all the lymphatic markers tested. Furthermore stimulation of PT and TD- LECs culture with the specific lymphatic endothelial growth factors VEGF-C induce proliferation on both PT and TD LECs culture but not of HUVECs indicating the presence of functional VEGFR-3 molecules during all the period of cultures on the LECs surface. As suggested by Podgrabinska et al, (2002) the different markers expression reflects the existence of LECs with different phenotypes in lymphatic macro- and microvessels, that results in different biological activities and functions. This hypothesis is supported by studies performed on BECs, that exhibit phenotypic and functional differences depending on their origin (adult versus foetal), anatomic localization, and vessels size (large versus capillary vessel) (Turner et al, 1987; Page et al, 1992), and that show different functions. In conclusion, our results collectively show that palatine tonsils are an important and easy to get source for isolating human lymphatic micro vessel ECs. Culture of PT- and TD-LEC in the presence of specific growth factors and extracellular matrixes was possible without losing their differentiated properties and functional activity. PT-LECs and TD-LECs may then represent new tools for investigating genetic, phenotypic and functional diversity between macro- and microvessels derived LECs isolated from different organs and tissue. Furthermore our work will contribute to determine some of the characteristic features of LECs, to understand their specialized lymphatic functions, to analyze their role in tumor lymphatic metastatic dissemination and to analyze their capability in supporting virus replication as already demonstrated for BECs (Lathey et al, 1990; Ricotta et al, 2001; Caruso et al, 2002).

REFERENCES

Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, and Jackson DG. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol* 144: 789-801, 1999.

Caruso A, Favilli F, Rotola A, Comar M, Horejsh D, Alessandri G, Grassi M, Di Luca D, and Fiorentini S. Human herpesvirus-6 modulates RANTES production in primary human endothelial cell cultures. *J Med Virol* 70: 451-458, 2003.

Gerli R, Solito R, Weber E, and Agliano M. Specific adhesion molecules bind anchoring filaments and endothelial cells in human skin initial lymphatics. *Lymphology* 33: 148-157, 2000.

Grinnel F. Fibroblast cell-substratum interactions: role of cold insoluble globulin (plasma fibronectin). *Experientia* 36: 505-507, 1980.

Jussila L, and Alitalo K. Vascular growth factors and lymphangiogenesis. *Physiol Rev* 82: 673-700, 2002.

Kahn HJ, Bailey D, and Marks A. Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcomas. *Mod Pathol* 15: 434-440, 2002.

Kleinman HK, Wilkes CM, and Martin GR. Interaction of fibronectin with collagen fibrils. *Biochemistry* 20: 2325-2330, 1981.

Kriehuber E, Breiteneder-Geleff S, Groeger M, Soleiman A, Schoppmann SF, Stingl G, Kerjaschki D, and Maurer D. Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. *J Exp Med* 194: 797-808, 2001.

Lathey JL, Wiley CA, Verity MA, and Nelson JA. Cultured human brain capillary endothelial cells are permissive for infection by human cytomegalovirus. *Virology* 176: 266-273, 1990.

Makinen T, Veikkola T, Mustjoki S, Karpanen T, Catimel B, Nice EC, Wise L, Mercer A, Kowalski H, Kerjaschki D, Stacker SA, Achen MG, and Alitalo K. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. *EMBO J* 20: 4762-4773, 2001.

Mancardi S, Stanta G, Dusetti N, Bestagno M, Jussila L, Zweyer M, Lunazzi G, Dumont D, Alitalo K, and Burrone OR. Lymphatic endothelial tumors induced by intraperitoneal injection of incomplete Freund's adjuvant. *Exp Cell Res* 246: 368-375, 1999.

Matsui K, Breitender-Geleff S, Soleiman A, Kowalski H, and Kerjaschki D. Podoplanin, a novel 43-kDa membrane protein, controls the shape of podocytes. *Nephrol Dial Transplant* 14 Suppl 1: 9-11, 1999.

Page C, Rose M, Yacoub M, and Pigott R. Antigenic heterogeneity of vascular endothelium. *Am J Pathol* 141: 673-683, 1992.

Petrova TV, Makinen T, Makela TP, Saarela J, Virtanen I, Ferrell RE, Finegold DN, Kerjaschki D, Yla-Herttuala S, and Alitalo K. Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J* 21: 4593-4599, 2002.

Podgrabinska S, Braun P, Velasco P, Kloos B, Pepper MS, Jackson DG, and Skobe M. Molecular characterization of lymphatic endothelial cells. *Proc Natl Acad Sci USA* 99: 16069-16074, 2002.

Ricotta D, Alessandri G, Pollara C, Fiorentini S, Favilli F, Tosetti M, Mantovani A, Grassi M, Garrafa E, Dei Cas L, Muneretto C, and Caruso A. Adult human heart microvascular endothelial cells are permissive for non-lytic infection by human cytomegalovirus. *Cardiovasc Res* 49: 440-448, 2001.

Turner RR, Beckstead JH, Warnke RA, and Wood GS. Endothelial cell phenotypic diversity. In situ demonstration of immunologic and enzymatic heterogeneity that correlates with specific morphologic subtypes. *Am J Clin Pathol* 87: 569-575, 1987.

Weninger W, Partanen TA, Breiteneder-Geleff S, Mayer C, Kowalski H, Mildner M, Pammer J, Sturzl M, Kerjaschki D, Alitalo K, and Tschachler E. Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. *Lab Invest* 79: 243-251, 1999

THE EUROPEAN JOURNAL OF lymphology and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

ASPECTS OF PREVENTION, DIAGNOSIS AND SURGICAL THERAPY OF LYMPHEDEMA SECONDARY TO CANCER DISEASES

Corradino Campisi

Department of Surgery – Section of Lymphatic Surgery and Microsurgery
S. Martino Hospital, University of Genoa, Italy
campisi@unige.it

Introduction

Chronic secondary lymphedema is a frequent complication of surgical treatment of malignant neoplasms, especially when radiotherapy follows[1].
The secondary lymphedema of the upper limb (post-mastectomy lymphedema) has an incidence, in patients who underwent axillary lymphadenectomy for breast cancer, between 5 to 25%, up to 40% after radiotherapic treatment[2].

Clinical Experience and Methods

We studied 50 patients with breast cancer. Patients were enrolled between April 1992 and June 1994, and treated in different Centers, besides ours, according to generally accepted oncological protocols. The inclusion criteria were the presence of invasive breast cancer (T1-T2 tumors), and a treatment plan that included axillary lymphadenectomy and irradiation.
The patients were divided in two groups of 25 each, comparable for age, sex, pathology and treatment, and followed up to 5 years after operation (18 lumpectomies and 32 mastectomy) for breast cancer (39 ductal and 11 lobular carcinomas). The number of excided lymphnodes varied from 12 to 26 (average 14). Radiation therapy was performed from the 3rd to the 6th week post-operatively.
One group of 25 patients was controlled only clinically (phisical examination, water volumetry) at 1-3-6 months and 1-3-5 years from breast cancer treatment. The other group of 25 patients was followed also by lymphatic scintigraphy performed pre-operatively and after 1-3-6 months and 1-3-5 years from operation.
In the first group, followed only clinically, lymphedema appeared in 9 patients after a period variable from 1 week to 2 years, with highest incidence between 3 and 6 months (time coincident with radiotherapy). We considered lymphedemas also at the very first stages (Ib-II), with a volumetric difference of 150 ml at least.
In the second group of 25 patients, lymphoscintigrapy showed pre-operative lymphatic circulatory alterations in 4 cases (reduced tracer transit capacity, tracer dispersion, decreased lymphnodal axillary captation, absence of deltoid way). Post-operatively, lymphatic impairment was found in 22 patients (5 cases after 1 month, other 3 cases after 3 months, 6 more at 6 months, other 5 at 1 year and, finally, 3 more after 3 years). In the remaining 3 patients, followed up to 5 years, lymphoscintigraphy did not point out any lymphatic alteration. All 22 patients underwent physical-rehabilitative preventive procedures[3]:

- For 2 weeks every month, for 3 months: manual lymphatic drainage (MLD) for half an hour, followed by moderate peristaltic mechanical lymph drainage (30-40 mmHg) for an hour, and again MLD for half an hour.
- After this treatment a functional elastic multilayer bandaging was used.
- During the 2 weeks free of treatment the patients wore only an elastic garement.
- The entire cycle was repeated another time for other 3 months.

This preventive therapeutic protocol allowed to have a clinically evident arm lymphedema only in 2 patients. These, who were not enough responsive to combined physical therapies (edema regression did not last long and there was the worsening of the edema), underwent early derivative lymphatic-venous microsurgery (at Ib and II stages)[4-5]. Microsurgery allowed to bring about a complete long term edema regression. The patency of lymphatic-venous anastomoses was proved by lymphatic scintigraphy also a long time after microsurgical operation (the last recent control was at 4 years from microsurgery so far)[6-7].

Results

The comparison of the two groups of 25 patients proved a statistically significant difference in the appearance of arm secondary lymphedema ($p = 0.01$, using Fisher's exact test). We can say, then, that the diagnostic and therapeutic above mentioned preventive procedures allow to reduce the incidence rate of lymphedema significantly, in comparison with patients who did not undergo this protocol of prevention[8].

Discussion and Conclusions

To this purpose, we do believe that to confirm this finding, it would be useful to conduct a multicenter study of the preventive protocol under the guidelines and the supervision of the ISL.

Due to the morbidity of lymphedema once it develops, prevention and information given early are extremely important. The earlier treatment begins after the appearance of lymphedema, the better is the prognosis for the patient.
Techniques of microsurgical lymphatic-venous anastomoses have been reported, both in the immediate post-mastectomy setting as prophylaxis and after the development of refractary lymphedema. The immediate and long-term results of these techniques are encouraging, with significant reductions in arm volume and improvement in symptoms that appear to be lasting. The proper indications for lymphatic microsurgery are patients in whom non-operative treatment, including physiotherapy, has failed to bring about at least a greater than 50% longlasting regression of limb edema.
In the prevention of lymphedema we could find an interesting role also of immunohistochemical studies of axillary lymphnodes and lymphatics[9]. This research entails an original classification of lymphatic and lymphnodal hystopathological alterations, concerning contractile and fibrose elements, described in arm lymphedema at different clinical stages. Similar findings have ben described in axillary lymphnodes after lymphadenectomy for breast cancer as if it simulated a condition of latent "idiopatic" arm lymphedema. We think that these data will help us in selecting patients with the risk of lymphedema who, thus, are subject to early preventive measures.

To conclude, an accurate early assessment of breast carcinoma patients plays a key role in the prevention, diagnostic evaluation and treatment of such a debilitating an distressing condition as lymphedema.

References

Campisi C. Il linfedema: aspetti attuali di diagnosi e terapia. Flebologia oggi 1997;1:27-42
Campisi C., Boccardo F., Casaccia M. Il linfedema dell'arto superiore secondario a linfoadenectomia e radioterapia adiuvante per cancro della mammella. Ospedali d'Italia Chirurgia 1997;3:112-118
Casley-Smith JR, Casley-Smith Judith R. Modern treatment for lymphoedema, 1994.
Campisi C. Boccardo F. Role of microsurgery in management of Lymphedema. Int. Ang.; 1999 Mar; 18(1): 47-51
Campisi C., Boccardo F., Alitta P. Derivative Lymphatic Microsurgery: indications, techniques and results. Microsurgery 1995;16,7:463-468
Campisi C. Lymphatic Microsurgery: legend or reality? Phlebolymphology 1994;7:11-15.
Campisi C. Boccardo F., Zilli A. Microsurgical treatment of peripheral lymphedema: an overview upon 25 years clinical experience. European Journal of Lymphology and Related Problems (EJLRP) 1997;7,26:36-40
Campisi C., Boccardo F., Borrelli V. et al. Linfedema dell'arto superiore secondario a trattamento per cancro della mammella: moderni aspetti di prevenzione, diagnosi e terapia microchirurgica. Clinica Chirurgica e Microchirurgia 1998;2:110-115
Dellachà A., Fulcheri E., Boccardo F., et al. Post-surgical lymphedema: iatrogenic or pre-existing disease? Lymphology 1998;31(suppl):562-565.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

SURGICAL PLANNING AND SURGERY IN THE ANIMAL ONCOLOGICAL PATIENT

Paolo Buracco
Faculty of Veterinari Medicine, Dipartimento di Patologia Animale, Grugliasco (Turin), Italy
paolo.buracco@unito.it

Surgery is the most important modality of treatment for most localized tumours since it results in a higher rate of cure than all other modalities. However, for most neoplasms, a multimodality approach is more often capable of reaching the best results in terms of disease free period and overall survival. Surgery should not always be extremely aggressive if other therapeutic tools (i.e., chemotherapy and/or radiotherapy) have shown to be effective for the control of that tumour. These ancillary treatments can be used preoperatively (neoadjuvant), postoperatively (adjuvant) or intraoperatively.
Cytology and histology are standard techniques to reach diagnosis in oncology. Diagnosis implies the primary tumour has been identified and that it has been ascertained if it has already spread regionally and/or sistemically. Diagnostic imaging techniques should be used properly to determine the extent and size of the primary tumour, mainly if surgical excision is going to be performed. Based on the standard biological-clinical behaviour of that tumour, metastasis is carefully looked for at the level of the regional lymph nodes, lungs, and/or other sites. If enlarged, regional lymph nodes are submitted to multiple fine-needle aspirates and/or biopsy but metastasis, even if present, may be missed. If endocavitary, surgical exploration may be indicated. Concerning lungs, metastasis evaluation implies right and left lateral and ventrodorsal views; in selected cases, a CT-scan may be useful. Bronchoalveolar lavage may be of some help for tumours (e.g. melanomas and some carcinomas) that have already reached a critical size; however, if metastasis is not demonstrated, doubt still remains. Other sites are evaluated depending on the standard clinical biology of that tumour (e.g.., bone metastasis in case of melanoma, carcinoma, osteosarcoma, etc). Attention is also driven to specific paraneoplastic syndromes that may accompany the neoplastic growth.
Some aspects must be kept in mind when a tumour surgical excision is planned: 1) knowledge of the standard biological behaviour of that tumour; 2) the first surgery has more probabilities to be successful; 3) resection of the tumour with uniform wide margins all around it may also result in the removal of the corresponding bone below the tumour; 4) this implies a correct planning of the aggressive surgery and reconstruction of the body part. The latter is better performed keeping in mind the surgeon's individual skills, all techniques of reconstruction, and the consequent functional deficit caused by the surgery performed. In general, limitations are mainly related to the probability of getting a definitive cure or, at least, to prolong dramatically his/her survival, preventing or retarding the metastatic spread; the latter is usually reached by the appropriate use of an adjuvant chemotherapeutic regimen.
Local recurrence is controlled through the application of a local treatment such as radiation therapy. In order to decide if a second surgery and/or adjuvant radiotherapy is indicated, infiltration of surgical margins has to be confirmed. Therefore, it should be emphasized the importance of identification of margins through the application of sutures or, more tipically, through the application of ink to all the bed where the suspicion of tumoral infiltration is real. Finally, a main concern in the oncological patient is, apart from the age of the animal that is not a limiting parameter if clinical conditions are good, a good quality of life of these patients after treatment.

Types of surgery in oncology and resection margins

Surgery may be classified as :
1) *diagnostic:* it is applied when less invasive procedures are not diagnostic. The goal is to get a sample of tissue to submit to cytology and histology. It is referred to as "incisional biopsy" (wedge of tissue obtained in an area easily removable in the subsequent resection of the tumour) and "excisional biopsy" (e.g., exploratory thoracotomy or laparotomy, or removal of cutaneous lesions surely benign);
2) *cytoreductive (debulking).* Inoperability may depend both on the type of growth (predominant infiltrative pattern) and tumour location (neck, axial skeleton, great vessels, nerves, etc, i.e. parts that cannot be radically excised without compromising some vital functions or life). Inoperable tumours may be debulked but some other therapeutic tools have proved to be effective in controlling their growth (radiation, chemotherapy);
3) *curative:* it provides an "en bloc" excision of the tumour with margins of 1-4 cm of normal tissue all around it; sometimes, depending on the neoplastic location, this is realized removing also the corresponding bone (mandibulectomy, maxillectomy, amputation, partial or total scapulectomy, pelvectomy, limb salvage, rib resection, etc).
4) *palliative:* it is rarely applied. It may be used if a real postoperative improvement of the quality of life of the animal is expected (e.g. pericardiectomy for tumours of the heart base).
Classification of surgical resection margins reflects all these different types of surgeries:
a) *intracapsular*: the mass is removed in pieces. This technique should be reserved only to benign lesions and it is obviously controindicated in case of malignant tumours but in some situations (e.g. malignancies extending in the nose, middle ear, spinal cord, etc) the surgeon is forced to remove the tumour in such a way ("debulking" or cytoreductive). Some adjuvant treatment is imperative since the tumour is macroscopically still present;

b) *marginal*: "shelling out" a soft tissue sarcoma is usually a very easy procedure but it is almost constantly followed by a recurrence since the dissection has been performed along the "pseudocapsula" that surrounds the neoplasm. The pseudocapsula is a result of the compression operated by the growing lesion and it is formed by normal tissue and neoplastic cells; a "satellite" (small nodule, possible result of an extravascular extension of the tumour in the reactive zone) and/or a "skip" metastasis (possible result of an intravascular metastasis in the normal tissue) can also be omitted. In order to avoid recurrences, wider margins of excision must be given involving also the surrounding normal tissues. This kind of dissection is correct only in case of confirmed benign tumours (e.g. lipoma);
c) *wide*: in this case the classic principle of the oncological surgery is theoretically respected and the tumour is removed with geometric appropriate margins. However, a "skip" metastasis may be omitted. Despite the apparently wide margins, the predominance of soft tissues in certain regions does not ensure a "wide margin excision"; besides, the nature of the soft tissue involved (muscle, fascia, ligaments, vessels, nerves, subcutis, etc) may reflect a different resistance to the neoplastic invasion. This should be taken in account during the tumour excision, together with the macroscopic extent of the lesion built up through some sophisticated imaging technique (CT-scan, MRI);
d) *radical*: it is mainly indicated for very malignant tumours; the most classical example is amputation.

Lymph node removal

If metastasis is demonstrated, regional lymph node(s) should be removed but if further lymphatic stations appear to be involved, treatment should be decided on an individual basis since prognosis in these cases is worse. If the metastatic lymph node is fixed to the surrounding tissues (first station only), an "en bloc" excision combined with an adjuvant treatment may be indicated. When metastasis is not demonstrated but regional lymph node(s) is/are enlarged, it/they are removed to clinically stage the tumour (histological evaluation).

Reconstruction

The first principle is that it is essential to remove any part suspected to be or contain tumour. This should be done the *first* time. The second principle is that it is better to allow a second intention healing rather than leaving some tissue because its removal complicates the primary closure of the wound. Several techniques provide the possibility of reconstruction when an aggressive removal does not allow a primary direct closure. These procedures are referred to as "tension-relieving techniques", "local flaps", "distant flap techniques", "axial pattern flaps", "free grafts", "myocutaneous and muscle flaps", etc. In many situations it may be advisable to utilize the "omentalization" technique to provide some more tissue protection, immunulogical defenses, and a support for an early granulation tissue formation.

Lung metastasis removal

Surgical removal of lung metastasis should be reserved to cases in which 1 or 2 lung metastatic nodules characterized by a long doubling time derive from a low-grade primary tumour. A prolonged disease-free interval should be expected after their removal. Adjuvant chemotherapy should be used in order to control further metastatic development.

Oncological surgery: general guidelines

- Use cryosurgery for very small epithelial tumours or when the anesthetic risk is really too high.
- Do not use local anesthesia to remove tumours: you will work badly and the injection may spread the tumour. However, a regional anaesthesia may be used when indicated (critical patient).
- In general, keep in mind the Halstead's principles when performing your surgery.
- *The bioptic site must be removed "en bloc".*
- Drape skin incisions to limit tumour implantation.
- Use surgical instruments as much as possible and not hands that may spread the tumour. Change surgical gloves whenever appropriate. Do not grasp the tumour with forceps: again, this may cause tumour spread.
- For the dissection use the scalpel as much as you can; scissors may be used to dissect between different planes. Complementary modalities such as electrocoagulation and laser can be used but they may complicate identification of the tumour margins (burnt tissue)
- Ligate or fulgurate early all the tributary vessels (firstly the veins).
- For sutures it is better to use monofilament than braided material in order to limit adhesion for neoplastic cells.
- Even if controversial for the possibility of spreading tumour cells, perform the wound lavage removing as much fluid as possible. In such a way, blood clots and tissue debris, potentially bearing some neoplastic cells, are removed.
- Do not use for the reconstruction the same surgical instruments, drapes and gloves used to remove the tumour.
- When the tumour has been removed, check that the margins are macroscopically appropriate. At this point you can also perform an intraoperative cytological examination.
- *Submit always the mass for histopathology.*
- *Always:* identify margins for the pathologist and have them carefully examined.

Prognosis

Prognosis in oncology involves the evaluation of the overall survival of the patient from the initial diagnosis (also when no treatment is applied) or from the beginning of the treatment (disease free and/or cure rate). Prognosis is not a constant and linear parameter since it can be favourably or unfavourably influenced by other factors, not dependent on the neoplastic growth.

Tumour-related prognostic factors

- Anatomical site of growth: important mainly for localized solid tumours. It may influence prognosis in terms of :
surgical approachability (see previously), also depending on a proliferative vs. infiltrative pattern of growth
different clinical biological behaviour of some tumours depending on their location in the body. Some classical examples are canine melanomas which are always malignant when

localized in the mouth and relatively benign when they grow on the flank; canine oral squamous cell carcinomas tend to be more benign when localized rostrally, more malignant when localized aborally;
to some extent, a worse prognosis may rise from *clinical complications* such as stenosis, compression, hemorrages, infections, functional deficits, etc caused by the neoplastic growth.
- Clinical staging of the tumour: a specific TNM clinical staging is established through a meticulous diagnostic work-up. Universally applied both in human and veterinary oncology, it facilitates the exchange of information among centres, and comparison of results obtained through different treatment protocols. The TNM system is based on: a) *T*: extension of the primary tumour; b) *N*: regional lymph node(s) status; c) *M*: presence or absence of distant metastasis, mainly in lungs. Other factors are: d) *P*: it is referred to as histopathological extent (e.g. in the thickness of the wall of a cavitary organ); e) *G*: it expresses the malignancy grade (low, medium, high); f) *L*: it expresses the tendency of the tumour to invade lymphatics; g) *V*: it express the tendency of the tumour to invade veins.
- Disease-related factors: such as the paraneoplastic syndromes (hypoglycemia, hypercalcemia, hyperthyroidism, fever, anemia, leukocytosis, DIC, gammopathies, degranulation effects of malignant mast cells, hyperthrophic osteopathy, cachexia, neurologic manifestations, etc). They can cause more morbidity (and eventually mortality) than the primary tumour.
- Chemosensitivity / Radiosensitivity: the first is influenced by tumoral cellular heterogeneity and growth rate. Concerning radiation, tumours that were considered in the past as tipically radioresistant may now be approached through a combination of surgery and radiotherapy with a dramatic improvement of control and overall survival (e.g. soft tissue sarcomas, nasal tumours, etc).

Patient-related prognostic factors
- Pre-existing diseases: cardiopathies, liver and renal diseases, etc, may make prognosis worse since they may limit the possibility of planning any treatment.
- Immunosuppressive treatments: in general, prolonged administrations of steroids facilitate tumour growth. This should be avoided if the intention is to cure the animal unless the tumour is a lymphoproliferative disorder (combination chemotherapy). In case no treatment is attempted, steroids may improve the quality of life in terminal patients, with the awareness of a shorter survival.

SELECTED REFERENCES

Withrow SJ e MacEwen EG. *SMALL ANIMAL CLINICAL ONCOLOGY*. WB Saunders Co., Third edition, 2001
Morris J e Dobson Mj. *ONCOLOGIA CLINICA DEL CANE E DEL GATTO*. Italian edition of Buracco P. UTET, 2003.
Pavletic MM. *ATLAS OF SMALL ANIMAL RECONSTRUCTIVE SURGERY*. WB Saunders Co., Second edition, 1999.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

NEW APPROACHES OF NEOPLASTIC DIAGNOSIS AND STAGING IN SMALL ANIMALS

Giorgio Romanelli DMV, Dipl ECVS
Clinica Veterinaria Nerviano
Via Lampugnani, 3
20014 Nerviano (MI)

giorgioromanelli@aliceposta.it

Diagnosis and instrumental staging

The biggest impact in veterinary oncological diagnosis is the wider introduction of sophisticated instruments like CT and MRI that due to the lowering of the cost are more easily available.

Helical TC, in particular has the possibility to perform a whole body scan in seconds and also the thoracic area can be clearly visualized.

The greatest sensibility of TC in respect to conventional X Rays allows the detection of pulmonary masses less than 1.5 mm in diameter in respect to the spatial resolution of 5-6 mm required by the traditional X-Rays study.

In the study of the abdominal cavity helical CT is better than radiography and, for many point of views, US .

In particular, the study of hepatic masses allows, even trough the contrast MPR reconstruction, to correlate the ratios between the lobe or the lobes with the hilar vascularization, determining the resecability.

In case of adrenal tumors, the visualization of a possible vascular invasion from a neoplastic thrombus and the empty part of the caudal vena cava, allows the planification of vascular incision or resection.

The presence of a satellite lymphanenopathy is easily visible and measurable.

CT is one of the best methods for the diagnosis of cutaneous and subcutaneous neoplasms, being in grade, better than any other instrumental techniques, to demonstrate the invasion of surrounding tissue and the presence, over all for sarcomas, of fascial infiltration and skip metastasis.

In the study of tumors of the CNS, CT, although not reliable as MRI, allows to discover the most part of space occupying lesion, cerebral or spinal.

Lymphnodes

Detection of the draining lymphnode, overall for head and neck cancer, can be obtained using a gamma camera, that is impossible for us at the moment, injecting micro bubbles in the tumor detecting them using US or trough a vital stain, usually metilene blue, injection in the tumor.

This last technique, even if very simple and promising, has many pitfalls.

In fact if the search is made too early the stain doesn't have the time to reach the node, if is made too late, there is a cascade effect and more than one node is stained.

During oncological surgery the draining lymphnodes are usually removed for staging trough the same incision or using a second approach.

Laparoscopy and Thoracoscopy

Thoracoscopy and laparoscopy have introduced even in veterinary medicine the concept of minimally invasive approach and the possibility to obtain a complete search of the abdominal and thoracic cavity.

Trough a very small access, usually less than 1 cm, a rigid scope connected to a camera is introduced in the cavity and, after a complete inspection, two or more instruments can be bring in and used for palpation, biopsy or surgical removal.

A new technique is the so called "laparoscopic second look" and can be used after tumor removal and chemotherapy to check the real value of medical intervention.

Cistoscopy

New optical instruments of very small diameter allow the inspection and the biopsy of some inaccessible organs like the male bladder.

In the female dog using a rigid scope 2.7 to 4 mm in diameter, allows the complete inspection of the urethra and the bladder and a biopsy can be performed passing a 1.2 mm forceps trough the instrument channel.

This technique is much less dangerous than fine needle aspirate trough the bladder wall, because there is the documented risk of neoplastic seedling in the abdominal wall

In the male dog, is impossible to use a rigid scope, because of the length of the urethra and the presence of the penis bone and a flexible scope, 2.5 mm in diameter with a working channel of 1.2 mm is used.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

STATE OF THE ART ON LYMPHATIC CANCER METASTASIS IN DOMESTIC ANIMALS

Attilio Corradi, Anna Maria Cantoni
Dpt. Animal Health, Faculty of Veterinary Medicine, University of Parma, Italy
e-mail: attilio.corradi@unipr.it

LYMPHATIC FUNCTION

The lymphatic circulation transports interstitial tissue fluid, macromolecules and cells back into the blood circulation. The lymphatic system begins with initial finger-shaped lymphatic vessels consisting of valveless lymphatic capillaries and precollectors with valves. The precollectors become collectors and transport the lymph to the so-called lymph stems. These vessels lead into the right and left lymphatic duct and into the jugular trunk of the head and neck region. At the end they join into the blood circulation at the junction point of the jugular and subclavian veins (2,3,4)

LYMPHATIC SYSTEM AND CANCER

The lymphatic system is one of the primary route for the neoplastic metastasis. Neoplastic cells spread via lymphatic vessels to the regional lymph nodes which play an important role in diagnosis, staging, and therapy. Epithelial malignant cells often metastasize to the regional lymph node and their presence are an important prognostic factor. There is a consequential anatomic progression of neoplastic cells from the original mass to the regional lymph node via lymphatic capillaries and collecting trunks. Once neoplastic cells are established in the sentinel lymph node further metastasis to lymph nodes occurs and then via lymphaticovenous connections to the general systemic circulation. Direct hematogenous metastasis may occur without regional lymph node involvement.
Lymphatic vessels containing clusters of neoplastic cells are often observed at the periphery of malignant neoplasia, but no evidence of metastasis has been observed in intratumoral ones.
Recent studies reported intratumoral lymphatic vessels in some neoplasia but it is not still clear if they are preexisting lymphatic vessels or the expression of lymphangiogenesis inside the neoplasia. The significance of preexisting peritumoral lymphatics as via for neoplastic cell metastsis is clear, but it is not still so clear whether neoplasia can stimulate lymphangiogenesis and whether neoplastic metastasis is mediated by molecular activation of the lymphatic system. Morphological transendothelial migration of neoplastic cell remain unknown and a transcellular passage, macrophage via, or channel-like path, polynucleated leukocytes via, have been hypothesized. Recently, several novel molecules have been identified that allow a more precise distinction between lymphatic and blood vascular endothelium.

MOLECULAR REGULATION OF TUMOR LYMPHANGIOGENESIS AND LYMPHATIC METASTASIS

These include VEGFR-3 (FLT-4),the receptor for the vascular endothelial growth factors VEGF-C and VEGF-D podoplanin, a glomerular podocyte membrane mucoprotein and the homeobox gene product Prox-1 that is involved in regulating development of the lymphatic system. A novel hyaluronan receptor termed LYVE-1 has been shown to be restricted to lymphatic vessels in normal tissue and associated with tumors (1,8,10,).Vascular endothelial growth factor-C (VEGF-C), a member of the VEGF family of growth factors stimulates lymphangiogenesis in addition to angiogenesis. The specific effects of VEGF-C on lymphangiogenesis depend on its proteolytic processing. The mature form of human VEGF-C stimulates both VEGFR-2 and VEGFR-3 and can therefore stimulate both angiogenesis and lymphangiogenesis, whereas the partially processed form preferentially bind and activates VEGFR-3 and specifically stimulates lymphangiogenesis. Another recent study demonstred the important role of VEGF-D in tumor lymphangiogenesis and metastasis. VEGF-C may induce the proliferation of lymphatic vessels in the stroma of primary gastric carcinoma, CD44v5 and CD44v6 positive, via activation of VEGFR-3, expressed on the endothelial cells of lymphatic vessels (5, 9,11)

CD44 AND METASTASIS

Active migration of tumor cells in extracellular matrix (ECM) is a prerequisite for tumor-cell invasion and metastasis. Specific membrane glycoproteins termed cell adhesion molecules, in addition to their basic role in cell-cell contact or cell-matrix interaction, were recently shown to be involved in more complex intracellular events, such as cell motility and gene transcription. CD44 is one of the adhesion molecules, and importance with respect to tumor-cell invasion and metastasis has become increasingly clear. CD44 is a type I transmembrane protein and functions as the major cellular adhesion molecule for hyaluronic acid (HA), a component of ECM. The extracellular domain (ectodomain) of CD44 interacts with ECM, and the intracellular domain associates with the actin cytoskeleton via binding to ERM (ezrin, radixin, and moesin) proteins. Thus, CD44 is an important mediator in regulating interaction between ECM and the intracellular actin cytoskeleton. CD44 is expressed in most human and dog cell types and is implicated in a

wide variety of physiological and pathological processes, including lymphocyte homing and activation, wound healing, and cell migration, as well as tumor cell growth and metastasis. The proteolytic cleavage of membrane proteins including CD44 has recently emerged as a key mechanism underlying their functional regulation.

CD44 IMMUNOPHENOTYPE ISOFORMS (v5-v6) IN GASTRIC CARCINOMA AND IN *HELICOBACTER PYLORI* GASTRITIS IN DOG

In normal gastric mucosa as well as in gastric carcinoma and hyperplastic gastritis associated to *H. pylori* infection no molecular CD44std. CD44v5. CD44v6, have been detected. A strong CD44 v5 immunostaining has been detected on the surface of neoplastic epithelial cells of dogs with gastric tumor in stomach mucosa as well as in neoplastic cell migration invading stomach wall and lymphatics vessels while CD44 v6 did not shown any immunoreaction differences in comparison to stomach of controls and gastritis ones (5,6).
CD44 v5 expression in canine stomach cancer suggests that it plays an important role in early stage of malignancy as well as in cell invasion but does not reveal expression differences in normal canine gastric mucosa as well as in canine gastritis induced by spontanueos *H. pylori* infection (6). As in human beings CD44 v6 expression in canine *H. pylori* gastritis has not shown immunoreactivity (6)..

GALECTIN 3 AND METASTASIS

The endogenous _-galactoside-binding protein galectin 3 is a member of a growing gene family of widely distributed carbohydrate-binding proteins that have been implicated in cell growth, di.erentiation, adhesion, and malignant transformation. Galectin-3 plays a role in cognitive cellular interactions during transformation and metastasis and occurs in increased amount in a wide variety of neoplastic cells. Over expression of galectin-3 was reported in gastric and colorectal carcinoma.

GALECTIN 3 EXPRESSION IN CD44 v5 IMMUNOPOSITIVE CANINE GASTRIC CARCINOMA

Normal epithelial cell of canine gastric mucosa as well as in chronic non-ulcerative canine gastritis by Helicobacter spp. did not express galectin-3 nor CD44v5. CD44v5 immunostaining was detected on the surface of the neoplastic epithelial cells of canine gastric carcinoma as well as in neoplastic migrating cells, while galectin-3 labelling was principally seen in the citoplasma of invasive neoplastic cells. A cytoplasmatic CD44v5 and galectin coexpression was only observed in the cytoplasm of invasive neoplastic cells in submucosa and muscularis as well as in lymphnodes (7). Findings confirm CD44v5 as a biological marker specific for malignant transformed epithelial cells in canine gastric carcinoma. CD44v5 and galectin-3 colocalization in invasive neoplastic cells suggests galectin 3 as a critical determinant for the metastasis (7).
A hypothesis for supporting this theory is that galectin-3 enhances metastasis of neoplastic cells functioning as survival antiapoptotic factor against cytotoxic reactive nitrogen and oxygen species generated when neoplastic cells are arrested within the microcirculation (7).

REFERENCES

1. Achen MG.et alii. (2000) Monoclonal antibodies to vascular endothelial growth factor-VEGF receptor-2 and VEGF receptor-3. Eur. J. Biochem. 2505-2515.
2. Azzali G, Orlandini G, Bucci G. (1989) Morphological characters of. the absorbing peripheral lymphatic vessel by TEM, SEM and three-dimensional models. Prog Clin Biol Res.; 295:487-92.
3. Azzali G. (2003) Transendothelial transport and migration in vessels of the apparatus lymphaticus periphericus absorbens (ALPA), Int Rev Cytol.;230:41-87.
4. Azzali G. (2003) Structure, lymphatic vascularization and lymphocyte migration in mucosa-associated lymphoid tissue, Immunol Rev.;195:178-89.
5. Corradi A. et alii. (1998). Expression of alternative spliced CD44 (isoforms v5-v6) transcript in canine gastric carcinoma. 16th Meeting of the European Society of Veterinary Pathology, Lillehammer (Norway) (vol. 16, pp. 27).
6. Corradi A. et alii. (1999). A comparative study on CD44 isoforms (v5-v6) in canine gastric carcinoma and in chronic canine gastritis by Helicobacter spp. 17 th Meeting of the European Society of Veterinary Pathology, Nantes (France). (vol. 17, pp. 164).
7. Corradi et alii (2002) Galectin 3 expression in cd44 v5 immunopositive canine gastric carcinoma 20 th Meeting of the European Society of Veterinary Pathology, Turin (Italy). (vol. 20, pp. 63).
8. Skobe M. et alii (2001) Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat. Med. 7: 192-198.
9. Stacker SA. et alii (2001) VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat. Med. 7: 186-191.
10. Swartz MA. et alii (2001) Lymphatic function, lymphagiogenesis and cancer metastasis. Microsc. Res. And Tech. 55: 92-99.
11. Yonemura Y. et alii (2001) Lymphangiogenesis and the vascular endothelial growth factor receptor (VEGFR)-3 in gastric cancer. Eur. J. Of Canc 37: 918-923.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No 43 • 2005
INDEXED IN EXCERPTA MEDICA

LYMPHATIC AND NEOPLASTIC METASTASIS: THE NEW ANATOMICAL CONCEPTS OF THE STUDY OF THE LYMPHATIC DRAINAGE OF VISCERAS EXPLAIN THE INVOLVEMENT OF REGIONAL NODES BY CANCER. A PERSONAL EXPERIENCE UPON 1000 CADAVERIES INJETIONS

A. PISSAS, F. BONNEL, J.B. DUBOIS

Department of visceral surgery General Hospital Louis Pasteur 30200 Bagnols sur Cèze. France Faculty of Medicine of Montellier 34000 Montpellier France Anti Cancerous Center of Montellier 34000 France

After 30 years of studying the lymphatic vessels on human beings we injected more than 1000 cadavers for the the interpretation of lymphatic metastasis on gastric, pancreatic, thyroid, oesophagus cancers.
For this injections we used special dyes (non vital staining dyes): coloured cedar oil according to Papamiltiades or a personal technique using China wood oil We found the different roads from the initial sub-serous lymphatic vessels towards the regional lymph-nodes and have described them like the classical authors.
But we had essentially insisted upon the fact that anatomical study of lymphatic drainage of corpus is a non sense because it concerns cadavers without life and physiology.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

TRANSMURAL PRESSURE IN INITIAL SUBPLEURAL LYMPHATICS DURING SPONTANEOUS OR MECHANICAL VENTILATION

Negrini Daniela 1, Moriondo Andrea 1, Mukenge Sylvain 2

1 Università degli Studi dell'Insubria, Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Varese, Italy 2 Ospedale San Raffaele, Dipartimento di Scienze Chirurgiche, Milano, Italy

Daniela.Negrini@uninsubria.it

Introduction. An important factor in enhancing lymphatic function is tissue movement, which determines cycles of external compression/expansion of the lumen of the initial lymphatic vessels. As a result, pressure gradients develop across the lymphatic vessel wall favouring both fluid entrance from the interstitium into the lymphatic lumen and/or progression of the newly formed lymph from one functional unit (lymphangion) to the next. At present, the effect of cyclic cardiac and respiratory movements on lymph formation in the thoracic tissues is unknown. Therefore, the aim of the present research was to measure the hydraulic pressure in the initial lymphatics (Plymph) draining the thoracic tissues and in the adjacent intercostal interstitial space (Pint) during spontaneous breathing or passive lung inflation.
Matherials and Methods. The role played by the mechanical tissue stress in supporting lymph formation and propulsion in thoracic tissues was studied in deeply anesthetized rats (n = 13) during spontaneous breathing or mechanical ventilation. After arterial and venous catheterization and insertion of an intratracheal cannula, fluorescent dextrans were injected intrapleurally to serve as lymphatic markers. After 2 hours from the dye injection, the intercostal lymphatics were visualized through fluorescent stereomicroscopy and the hydraulic pressure in lymphatic vessels (Plymph) and adjacent interstitial space (Pint) was measured by micropuncture.
Results. During spontaneous breathing, end-expiratory Plymph and corresponding Pint were - 2.5 ± 1.1 (1 SE) mmHg and 3.1± 0.7 mmHg ($p < 0.01$) and dropped to -21.1 ± 1.3 mmHg and -12.2 ± 1.3 mmHg, respectively, at end-inspiration. During mechanical ventilation with air at zero end-expiratory alveolar pressure, Plymph and Pint were essentially unchanged at end expiration, but, at variance with spontaneous breathing, they increased at end-inspiration to 28.1 ± 7.9 mmHg and 28.2 ± 6.3 mmHg, respectively. The role of the tensile and/or compressive tissue stresses during active or passive chest expansion was evaluated from hydraulic transmural pressure gradient DPtm (DPtm = Plymph – Pint) developing during the respiratory cycle across the lymphatic vessel wall. During spontaneous breathing DPtm was in favour of lymph formation throughout the whole respiratory cycle (DPtm = ~ -6.8 ±1.2 mmHg), while it was essentially nullified (DPtm = ~ - 1.1 ± 1.8 mmHg) during mechanical ventilation.
Conclusion. Local tissue stress due to active contraction of inspiratory muscles is required for an efficient lymphatic drainage from the thoracic tissues. Given the importance of the lymphatic system in controlling fluid homeostasis in the thoracic tissues and in particular in the pleural cavity, mechanical ventilation "per se" is therefore expected to determine an increase of thoracic tissues hydration and pleural effusion.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

EMILINs EXPRESSION IN LYMPHATIC SYSTEM

Carla Danussi, Paola Spessotto, Bruna Wassermann, Francesca Merlo, Roberto Doliana, Alfonso Colombatti
CRO-IRCCS, Division for Experimental Oncology 2, Aviano (PN), ITALY
carladanussi@yahoo.it
Key words: EMILINs, Lymphatic endothelial cells, Lymphangioma.

Introduction: EMILINs (Elastin Microfibril Interface Located proteIN) are a family of extracellular matrix proteins characterized by a peculiar domain structure, including a N-terminal cysteine-rich domain, called the EMI-domain, followed by a coiled-coil domain and a gC1q-like domain, (Colombatti A et al., 2000). The first isolated member of the family is EMILIN1, a glycoprotein particularly abundant in aorta and present in connective tissue of a wide array of organs mainly in association with elastic fibers (Doliana R et al., 1999). The family also includes EMILIN2 (Doliana R et al., 2001), Multimerin1, a protein secreted by endothelial cells (EC) and platelets (Hayward CP et al., 1995) and Multimerin2, a pan-EC surface glycoprotein (Christian S. et al., 2001).
KO mice for EMILIN1 gene have been already established and they display mild elastogenesis and vascular cell defects (Zanetti M et al., 2003). However, at the present the precise function of EMILINs is unknown. Interestingly in a comparative microarray analysis of gene expression profiles of lymphatic endothelial cells (LECs) and vascular endothelial cells (EC), EMILIN1 has been found to be selectively and abundantly expressed in LECs, (Podgrabinska et al., 2002). Accordingly to this finding, we hypothesized an involvement of EMILINs in the lymphangiogenesis. To investigate a possible role of EMILINs in this context we analysed inducted lymphangiomas in wild type and EMILIN1 KO mice.
Materials and Methods: CD1 wild type and EMILIN1 KO mice were intraperitoneal injected with incomplete Freund's adjuvant for the lymphangioma induction and LECs were isolated from the tumor masses (Mancardi S et al., 1999). The LECs expression of EMILINs was detected by RT-PCR. Immunofluorescence (IF) techniques on cultivated LECs and on cryostate tumor sections were applied to study the in vitro production of ECM proteins and to visualize the distribution of EMILINs in lymphatic vessels.
Results: the RT-PCR analysis reveal that LECs express EMILIN1 and EMILIN2 mRNA, whereas Multimerin2 mRNA is not detected. The data about EMILIN1 mRNA expression is supported by the IF results. In cryostate sections EMILIN1 surrounds lymphatic vessels, visualized by a double staining with an anti LYVE-1 antibody and in the inner tumour mass EMILIN1 is overexpressed. Interestingly Multimerin2 stains selectively vascular EC. Whereas there is no significant difference in the development and in the size of the lymphangiomas inducted in wild type and EMILIN1 KO mice, preliminary data show that the lymphatic tumour vascularization seems to be different in the two types of mice.
Conclusion: The strictly association between EMILIN1 and lymphatic vessels suggests a structural role of EMILINs in the architecture and development of the lymphatic system.
Further studies will be addressed to confirm the preliminary observation of a different pattern of lymphatic vascularization observed in the lymphangioma developed in wt compared to EMILIN1 KO mice.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

ULTRASTRUCTURAL MODIFICATIONS OF INTESTINAL LYMPHATICS DURING OVINE PARATUBERCULOSIS

Giacomo Rossi, Chiara Tarantino, Michela Gregori, Giovanni Braca, Ennio Taccini.
Dipartimento di Scienze Veterinarie, Università di Camerino, Via Circonvallazione 93/95 - 62024 Matelica (Mc).
giacomo.rossi@unicam.it

Key words: lymphatics vessels, ultrastructure, alterations, paratuberculosis, sheep.

One of the most important pathological findings in regional enteritis due to Mycobacterium avium subsp. paratuberculosis infection in sheep is represented by the severe lymphatic enlargement with alteration in normal drainage of the lymph in affected areas. Pathological alterations of lymphatic vessels of the submucosal areas are observed already in the very early stage of infection, associated with moderate mono and polymorph nuclear cells infiltration of the mucosa. To verify the most important ultrastructural changes in normal morphology of lymphatic during the disease, and to evaluated the contribute of these alterations to the development of the pathology, the fine structure of intestinal lymphatics in 14 affected and sero-microbiologically positive sheep and 4 unaffected controls was performed. Although obstructed lacteals are considered to be of relevant pathological finding, no detailed electron microscopic studies in lymphatic capillaries in this diseases could be found. The most relevant observation in affected animals were the presence of only closed intercellular junctions in mucosal and submucosal lacteals. In addition, only in areas of regional enteritis a heavy accumulation of protein rich lymph in the abluminal surface of lymphatics was consistently seen. These alterations indicate the decreased permeability of the lymphatic wall as an early pathological event during the disease, and this permeability reduction could be the major event in the development of submucosal edema. In addition a decrease absorption of chilomicrons is hypothesized and may contribute to worsening of general conditions, diarrhoea and cachectic status of heavily affected animals.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No 43 • 2005
INDEXED IN EXCERPTA MEDICA

THE EVALUATION OF LYMPHOGRAPHY IN THE ABDOMINAL PATHOLOGY

Tatiana Jurgova, Tatiana Taseva, Jozef Radonak, Jan Danko, Marta Chylova, Igor Dranga

1.P.J.Safarik University, Radiodiagnostic Medicine Clinic ,Kosice,Slovakia
2.P.J.Safarik University, Chirurgical Clinic, Kosice, Slovakia
3.University of Veterinary Medicine, Department of Anatomy, Kosice, Slovakia
tjurgova@central.medic. upjs.sk

Key words: nodus lymphaticus

Introduction
On the level of pelvis minor and abdomen can be present changes in lymphatic system on the basis of diseases of different etiology. The reason for these changes can be inborn defects, parasitic, neoplastic diseases. On the other hand the lymphatic system can cause changes in the surrounding structures and in the main vessels. The changes in the lymphatic system can demonstrate itself in the blockade of lymphatic vessels, insufficient archaortic filling, and also in the leaking of contrast medium out of the lumen of the vessels.
Material and methods
116 patients were examined mainly with oncological diagnosis but also some patients who demonstrated diseases of unknown etiology. These patients were examined by USG, CT,MR, but the final diagnosis was stated only after lymphographic examination. We carried out bipedal lymphography by J.B.Kinmonth the patients were given 16 ml of contrast medium Lipiodol UF into a prepared lymphatic vessel in dorsum pedis. X-ray pictures were taken during the examination and after 24 hours in different projections.
Results
In patients with neoplastic and inflammatory processes we could see blockades of lymphatic vessels changes in the structure of nodes, pressure and infiltrative changes in main vessels. In patients with inborn anomalies were found eg lymphovenous junctions or leaking of contrast medium out of lymphatic vessels.
Discussion
For the correct interpretation of the X-ray picture and stating the correct diagnosis is necessary to have a thorough knowledge of the normal anatomy of the lymphatic system. Although these days the classic lymphography is a less used method, in some cases it helps to prove those changes which other modern non-invasive methods are not able to prove.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

OBSTRUCTIVE LYMPHEDEMA AFTER HYSTERECTOMY WITH ILIAC DISSECTION

Waldemar Lech Olszewski
Department of Surgical Research & Transplantology, Medical Research Center, Polish Academy of Sciences
wlo@cmdik.pan.pl

Key words: hysterectomy; lymphedema; lymphangiogenesis

Extirpation of iliac lymph nodes and vessels and subsequent radiotherapy are followed in patients with cervical cancer by development of lower limb lymphedema in a high percent of cases. Edema develops within 6 to 18 months after treatment. It affects thighs, then lower legs. Lymphoscintigraphy (Lsc) shows obstruction at the iliac level with dense collateral circulation around hip and in later stages dermal backflow in thigh and calf. Ultrasonography of iliac and femoral veins shows in 10-30% of cases obstruction or narrowing of venous lumen. Those who survive without cancer metastases reveal after 2-3 years obliteration of thigh afferent vessels and fibrosis of inguinal lymph nodes. The question arises whether the vessels seen on Lsc are new lymphatics formed after treatment or dilated preexisting callateral and why they become obliterated in course of time. Aim. The aim of studies was to compare the lymphangiographic and immunohistopathological pictures of thigh lymphatics and lymph nodes. Material and methods. Ten female patients with uterine cancer 6 months to 5 years after hysterectomy and radiotherapy with lymphedema of lower limb stage III-IV. Isotopic lymphangiography was performed followed by surgical revision of inguinal area and either lymph node-vein anastomosis or removal of fibrotic structures. Fragments of lymph node with afferent vessel were taken. Five female patients undergoing varicous veins operation with ligation of inguinal tributaries served as donors of lymph node and lymphatic vessel fragments. Results. On lymphoscintigraphy all patients revealed dilated network of lymphatics in thigh up to inguinal ligament level. The total area of opacified nodes was lower than on the contralateral non-swollen side. Stereoscopic specimens of skin lymphatics showed dilated subpapillary and papillary vessels. All lymphatic fragments were LYVE1, Prox1 and podoplanin-negative. Moreover, only slight staining for VEGF C and VEGFR 3 was seen on lymphatic endodothelium. Lymph nodes were depleted of lymphocytes. The sinuses stained positively for LYVE1. Control specimens did not show dilated lymphatics, whereas large collectors remained LYVE1-negative and VEGF C and R was only weakly expressed. Conclusions. No pictures of lymphangiogenesis were seen. Collaterals were most likely dilated pre-existing vessels. Lymphangiogenesis in adult humans differs from what has been described in mice. These animal findings should not directly be extrapolated to adult humans.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

ASPECTS OF THE IMAGISTIC PARTICULARITIES INDUCTED BY THE PHLEBOEDEMA ON THE FLABBY PARTS AT THE LEVEL OF THE INFERIOR MEMBERS

F.C. Rada, S. Motoi, S. Blaj, T. Mihoc, Lucia Parvan, I.O.Rada,
Romanian Society of Lymphology, Medical and Pharmaceutical University
florinrada@xnet.ro

Key words: Phlebedema, computerized tomographie, magnetic resonance images

The purpose of the research: there has been followed the identification of the imagistic particularities at the patients with phlebedema.
Material, method. The study was imagined and practiced at 21 patients with PTS (post- thrombosis syndrome), at 14 patients with hydrostatic varix (HV) and at 8 patients with profound acute thrombophlebitis (PAT). There have been followed the modifications on the CT scan and MRI generated by the alterations in the conjunctive tissue of the inferior members in the conditions of the venous haemodynamic modifications generated by the venous insufficiency (VI).
Concomitantly, there have been evaluated also the composition and the evolution of the edema liquid.
Results: CT scan and MRI have emphasized 2 groups of images at the level of the conjunctive tissue: tiger images present in the hypoderm are characteristic to the acute thromboplebitis. Honey comb images have not been identified at the patients with PTS (rule at patients with lymphedema). In the hypoderm of these patients there have been emphasized uniform structures (lypodermatosclerosis after the suppression of the fat tissue) and under aponevrose calcifications.
The macromolecules (proteins- lipids) have been in low percentage in the edema liquid of the patients with PTS (1/10, respectively 1/6 of the serum values) .
Conclusions: These results can facilitate the understanding and the completion of the aspects and physiopathologie knowledge , especially in the PTS case in the presence of some vast beaches of uniform structures(lypodermatosclerosis), without the necessity of the introduction of these explorations in the diagnosis algorithm.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

ROLE OF TRANSFORMING GROWTH FACTOR-ßETA, (TGF-ß,), IN TUMOR METASTASIS

Khaki Arash1, Nilforushan Nahedeh2, Khaki A.A 3, Rezaie Ali4.
Islamic Azad University,Dept of Veterinary Pathology Tabriz,IRAN,
e-mail: arashkhaki@canada.com

Introduction: Metastasis is a complex process caused by elaborate interactions between tumor cells and the surrounding tissues. We are seeking to understand the molecular and cellular mechanisms that allow primary tumors to form metastases. Metastasis the spread and growth of tumor cells to vital organs is the ultimate cause of death in the vast majority of cancer patients, and veins and circularly system have important role in tumor spreading.

Material and method: The role of transforming growth factor-beta (TGF-beta) signaling in such epithelial-mesenchymal interactions was determined by conditional inactivation of the TGF-beta type II receptor gene in mouse fibroblasts. Several lines of evidence suggest that transforming growth factor b1 (TGFb1), play a role in Tumor progression. TGFb1 inhibits the growth of normal epithelial cells; resistances to this inhibitory activity or proliferation in response to TGFb1 by neoplastic cells are mechanisms of tumor progression. TGFb1 has angiogenic properties, as well as the ability to increase the invasive and metastatic potential of neoplastic cells. Some of its immune regulatory properties such as inhibition of T-cell proliferation and deactivation of natural killer (NK) cells and macrophages, may enable tumor cells to evade immune surveillance.one of the other molecule that help to spreading TGFb1 is CD105 (endoglin). CD105 is a proliferation-associated and hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells (EC).

Result: It is a receptor for transforming growth factor (TGF) -ß1 and -ß3 and modulates TGF-ß signaling by interacting with TGF-ß receptors I and/or II. CD105 is able to be shed into the circulation, with elevated levels detected in patients with various types of cancer and positively correlated with tumor metastasis. Many organs tumor such as Lung, breast and prostate tumor cell lines can secrete TGFb1, and these cells frequently do not respond to TGFb1 or they can even be stimulated to proliferate. Recently, it has been reported in an immunohistochemical study on lung adeno carcinomas that TGFb1-positive staining of neoplastic cells could be an unfavorable prognostic factor. The loss of TGF-beta responsiveness in fibroblasts resulted in intraepithelial neoplasia in prostate and invasive squamous cell carcinoma of the fore stomach, both associated with an increased abundance of stromal cells. Activation of paracrine hepatocyte growth factor (HGF) signaling was identified as one possible mechanism for stimulation of epithelial proliferation.

Conclusion: Thus, TGF-beta signaling in fibroblasts modulates the growth and oncogenic potential of adjacent epithelia in selected tissues. We can conclusive some factors look like circularly factor: CD105, or immune factor: inhibition of T-cell proliferation, deactivation of natural killer (NK) cells and macrophages, epithelial factor: hepatocyte growth factor (HGF), have mainly and important role in TGF-beta metastasis in many organs in body.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

THE ROLE OF VEGF AND ITS RECEPTOR KDR IN PROMOTING TUMOR ANGIOGENESIS IN FELINE AND CANINE MAMMARY CARCINOMAS: A PRELIMINARY STUDY OF AUTOCRINE AND PARACRINE LOOPS

Francesca Millanta, Simonetta Citi.2, Chiara Vaselli 2, Alessandro Poli
Department of Animal Pathology ? Department of Veterinary Clinic, School of Veterinary Medicine, University of Pisa, Italy, 2 Private Practitioner, Pisa, Italy.
e-mail: millans@vet.unipi.it

Introduction: angiogenesis is regulated by specific angiogenic factors and their receptors in primary breast cancer; this phenomenon has shown to be of predictive value in primary breast cancer. Vascular endothelial growth factor (VEGF) and its receptor KDR constitute an important angiogenic pathway which is up-regulated in several human solid tumours. We therefore evaluated the angiogenenetic process in feline and canine mammary carcinomas and its prognostic potential. Materials and methods twenty-nine samples of canine and 48 of feline mammary tumours were investigated. The subjects were surgically treated and submitted to a two-year follow-up. The tisssue samples were investigated by immunohistochemistry to determine the expression of VEGF, of its receptor KDR and for the quantification of the microvessel density (mvd). These variables were related to some relevant clinicopathological parameters and to overall survival (os). VEGF and KDR expression were evaluated in epithelial, stromal and endothelial compartments in order to identify autocrine and/or paracrine loops. Results in dogs an increased VEGF expression did not show any statistical correlation with the clinicopathological parameters examined and was not correlated to a poorer prognosis. Mvd was found to be significantly correlated to the histologic type (p=0.04), age (p=0.041), tumour grading (p=0.02), and to the os (p=0.01).in cats VEGF expression was significantly correlated to tumor grading (p=0.01) and os (p=0.03), while no significant associations were found between mvd and the other parameters. VEGF and KDR were found to be detected on the epithelial, and/or endothelial and/or stromal cells of the carcinomas in both species, suggesting indications for some possibile autocrine and paracrine loops. Conclusion our results encourage further studies on the possible prognostic role of VEGF and mvd in canine and feline mammary tumours and on the role of growth factors and their receptors in promoting tumour proliferation and an "angiogenetic shift". The VEGF/KDR system may play a role in malignant transformation and tumor progression.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

THE EFFECT OF HUMAN SKIN LYMPH ON TUMOR CELL PROLIFERATION

Waldemar Lech Olszewski, Irena Grzelak
Surgical Research and Transplantation Department, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland, and Norwegian Radium Hospital, Oslo, Norway
wlo@cmdik.pan.pl

Key words: lymph; tumor; metastases; cytokines

Contemporary attention on tumor proliferation has been focused on lymphangiogenesis mediated by angiogenetic products of tumor cells. Neglected has been the humoral environment where tumor cells grow. Both primary and metastatic cells proliferate, at least in the initial stage, in tissue fluid (lymph) characteristic for the tissue in which they are located. The aim of this study was to examine the effect of normal human skin lymph on proliferation of rapidly growing, established, lymphoid and non-lymphoid human tumor cell lines which either grow or metastasize in skin and subcutaneous tissue. Human skin lymph displayed both inhibitory and stimulatory effects on proliferation of the examined tumor cell lines of lymphoid and non-lymphoid origin, both in short-term and long term cultures. The effect was characteristic for a particular tumor cell line. Low lymph concentrations (5 and/or 20%) stimulated colony formation in a soft agar of most of the examined cell lines: B cell lines: Rael by 39.6%, Raji by 30.0% and Balm1 by 197.4%; T cell lines: CCRF-CEM by 82.3%, CCRF-HSB by 204.4%, JURKA T by 72.6% and MOL T by 95.2%; melanoma: FMEX by 24.9% and LOX by 558.3%; sarcoma SAOS-2 by 37.2% and dermoid origin: HT3 by 52.2%. High lymph concentrations (80%) inhibited the growth of B cell lines: Rael by 29.5% and KM3 by 55.1 %, T cell lines: CCRF-CEM by 19.6% and CCRF- HSB by 23.7%; melanoma: FMEX by 8.8%; sarcoma: OHS by 60.5% and OAOS-2 by 24.8% and dermoid origin: HT3 by 42.8% and HACAT by 47.5%; whereas stimulated B cell lines: Balm1 by 33% and Raji by 10.9%; T cell lines: Molt-4 by 35.2% and JURKAT by 50.3% and melanoma cell line: LOX by 605.9%. The inhibitory effect of skin lymph was often more pronounced (as for FMEX and OHS tumor cells) when tumor cells were cultured in a serum-free system in a Waymouth medium. No significant differences between the results of short-term cultures and soft-agar colony formation cultures were observed. Preincubation of tumor cell s with lymph demonstrated that the growth regulatory lymph factor(s) might be absorbed by tumor cells. Our results indicate that the in vivo tumor growth in its tissue-specific humoral environment may have different kinetics than in the in vitro standard culture media.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

LYMPHANGIOGENESIS IN MAMMARY TUMOURS OF THE CAT ASSESSED BY VEGFR-3 EXPRESSION

Giuseppe Sarli, Barbara Brunetti, Antonio Rizzo, Cinzia Benazzi
Department of Veterinary Public Health and Animal Pathology, Bologna, Italy
e-mail: giuseppe.sarli@unibo.it
Key words: lymphangiogenesis, cat, mammary tumor, VEGFR-3

Introduction. Lymphatics play a crucial role in the formation of metastasis, and only recently were found specific markers, such as VEGFR-3 (Vascular Endothelial Growth Factor Receptor-3) that has been employed in this study. It is found in both lymphatic and blood vessels during embryogenesis, and only in lymphatics after birth. This study is aimed to verify if lymphangiogenesis develops (de novo or from pre-existing lymphatic vessels) in the intra(IT)/extratumoral(ET)stroma of a series of feline mammary tumours.
Materials and Methods. The samples were 6 cases of normal mammary gland (NMG), 10 benign (BN) and 32 malignant (MN) neoplasms, all formalin-fixed and paraffin-wax embedded. Malignancies were graded into non-infiltrating (stage 0) and infiltrating with stromal invasion (stage I) or lymphatic or blood emboli and/or regional lymph node metastases (stage II). More 4 ?m sections from the same samples were immunohistochemically (IH) stained with a laminin/VEGFR-3 double stain. Lymphatics, assessed as all vessels negative for laminin and expressing or non-expressing VEGFR-3, were counted in 10 intratumoral/intramammary(IT/IM) and 20 extratumoral /extramammary (ET/EM) fields. Counts included VEGFR-3 positive, VEGFR-3 negative and total (the sum of both) lymph vessels.
Results. A red intracytoplasmic staining revealed the anti-VEGFR-3 IH positivity, and a sub-endothelial or sub-epithelial (periductular and perialveolar) brown staining the anti-laminin positivity. In NMG, BT and MT, the positive or negative or total VEGFR-3 lymphatic vessels had a significantly higher number in the ET(EM) vs IT(IM) fields (Spearman test, $P<0.01$ for all comparisons). Comparing IM with IT fields no difference was detected in the number of lymphatic vessels, whereas there was a significantly higher number of total and VEGFR-3 negative (Spearman test, $P<0.05$), but not of VEGFR-3 positive lymphatics (Spearman test, $P=0.26$) in ET vs EM fields. No difference emerged comparing IT or ET counts among the 3 histological grades in MT, except for IT VEGFR-3 positive lymphatic vessels in stage II carcinomas vs stage 0 and I (Spearman test, $P<0.05$), which were more numerous in the former.
Discussion: The significantly higher number of lymphatic vessels in the ET vs IT stroma is likely to reflect the expansive growth of the tumour and the concentration of vessels in the extra(peri)tumoral areas. This is enforced by the significant increase in VEGFR-3 negative and total but not VEGFR-3 positive lymphatics in ET vs EM fields. In spite of an extremely limited lymphangiogenesis, early metastases of mammary carcinomas are located in the regional lymph node. This indicates that the neoplastic cells leave the primary tumour through pre-existing but not de novo lymphatics, and chemical means may attract neoplastic cells into the lymphatics.
Conclusions: In the comparison between malignancies and benign tumours and normal glands, the number of lymphatics is not increased. The known ability of carcinomas to an early spread via the lymphatics is not consequent on a real increase of lymphatics in the intra/extratumoral stroma, but it may depend on the ligand/receptor interaction between neoplastic cells and lymphatic endothelium.

THE EUROPEAN JOURNAL OF lymphology and related problems
VOLUME 15 - Sp Co Nr 43 - 2005

LYMPHANGIOTROPHISM OF MAMMARY CARCINOMAS OF THE CAT: ROLE OF VEGF-C AND CD-44

G.Sarli, L. Diracca, F.Sassi , B. Brunetti, C. Benazzi
Department of Veterinary Public Health and Animal Pathology, Bologna, Italy
e-mail: giuseppe.sarli@unibo.it

Key words: lymphangiogenesis, cat, mammary tumor, VEGFR-3

Introduction. Tumour dissemination through the lymphatics (Lfs) is well known in feline mammary carcinomas in which regional lymph node is the site of early metastases. VEGF-C increases vascular permeability and facilitates transendothelial neoplastic migration. CD-44 promotes cell migration inducing small gaps in the endothelial wall. This study is focused on the expression of VEGF-C and CD-44 in feline mammary samples to ascertain any correlation with the quantitative analysis of Lfs.
Materials and Methods. Immunohistochemistry (IHC) was applied to 4?m-thick sections of 48 samples of normal mammary gland (NMG), benign (BT) and malignant (MT) tumours, using anti-VEGF-C (clone Z-CVC7) and anti-CD44var (v5) (clone VFF-8) antibodies. The IHC expression of both VEGF-C and CD44 was analyzed with a 40x objective in five intratumoral(IT)(intramammary-IM) and 10 extratumoral(ET)/ (extramammary-EM) fields, and then evaluated in the epithelial adenomatous or carcinomatous component (absent=0; low=10; intermediate=100; high=1000).
Results.VEGF-C stained positive in the cytoplasm of endothelial and epithelial (normal, benign or malignant) cells, and CD44 only in epithelial cells. Comparing IT(IM) and ET(EM) VEGF-C expression, there was no difference in NMG, BT and non-infiltating MT, whereas a significant lower expression in ET compared to IT emerged in invasive MT ($P<0.05$ Spearman Test). Again, no difference was found in VEGF-C expression in NMG, BT and MT respectively in ET(EM) and IT(IM) fields. Comparing IT(IM) vs ET(EM), CD44 expressed a significant difference (lower values in ET) in BT and invasive MT ($P<0.01$ Spearman Test), but not in non-invasive MT and NMG. CD44 expression was increased only in the ET(EM) from NMG to BT to MT ($P<0.05$ Spearman Test). Comparing mean CD44 or VEGF-C expression with Lfs assessment from previous paper (Sarli et al., Lymphangiogenesis in mammary tumours of the cat assessed by VEGFR-3 expression, in this issue), a significant inverse correlation was found between CD44 expression in the epithelial cells and VEGFR-3 expression in lymphatic vessels in BT and invasive MT.
Discussion. Intratumoural high VEGF-C expression reflected the stronger need for angiogenesis in infiltrating MT than non-infiltrating MT and BT, but showed no correlation with lymphatics assessment. CD-44 expression, instead, was similar to VEGF-C in MT, but inversely and significantly correlated to lymphatics expressing VEGFR-3. Both molecules were not strictly associated to malignancy, but showed major differences in infiltrating MT. CD44, which can interact with vessel endothelium and has also a role in promoting cell-cell adhesion, is inversely associated with VEGFR-3 expressed in lymphatics of invasive MT. This may indicate that extratumoural fields, characterized by low CD44 expression and high numbers of VEGFR-3 positive lymphatics, are the site of neoplastic-endothelial cells interaction.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No: 43 • 2005
INDEXED IN EXCERPTA MEDICA

ENDOTHELIAL AREA AND MICROVASCULAR DENSITY IN A CANINE NON-HODGKIN'S LYMPHOMA: AN INTERSPECIES MODEL OF TUMOR ANGIOGENESIS

Nicola Zizzo 1 ,Rosa Patruno 2, Antonia Lionetti 1, Aldo Di Summa 1, Pantaleo Bufo 3, Antonio Pellecchia 2, Domenico Ribatti 4 and Girolamo Ranieri 2
1 Department of Animal Health and Well-Being, University of Bari Veterinary Medical School, Bari, Italy; 2 National Cancer Institute of Bari, Italy; 3 Department of Human Pathology, University of Foggia Medical School, Foggia, Italy; 4 Department of Human Anatomy and Histology, University of Bari Medical School, Bari, Italy
e-mail: n.zizzo@veterinaria.uniba.it
Key words: endothelial area, microvessel density, dog lymphoma

Introduction
Experimental and clinical data indicate that tumor progression is associated with angiogenesis and that increase in microvessel density (MVD) is associated with a poor prognosis, haematological malignancies. No data have been published concerning the relationship between angiogenesis and malignancy grade in canine non Hodgkin's lymphoma. In this study we have evaluated this relationship in a series of 43 cases of canine non Hodgkin's lymphoma.
Matherials and Methods
Canine NHL were selected and classified according to a modified Kiel classification. Briefly, 6-micrometer thick serial tissue sections were incubated with a rabbit polyclonal antibody anti factor VIII- related antigen (Dako, Glostrup, Denmark). The bound antibody was visualized by using a biotinylated secondary antibody, an avidin-biotin peroxidase complex, and 3-amino-9-ethylcarbazole. Differences in both MVD and EA between low-grade and high-grade canine NHL groups was assessed by t Student-test, correlation between MVD and EA was evaluated by Pearson test.
Results
MVD mean was significantly higher in high-grade (23±9 s.d. at 400x and 8±3 s.d. at 1000x) than in low- grad (7±4 s.d. at 400x and 3±2 s.d. at 1000x) canine NHL. Significant differences in EA means were found between low-grade (78.33 x 10-2 ?2 ± 20.62 s.d. at 400x and 23.68 x 10-2 ?2 ± 7.67 s.d. at 1000x) and high-grade (116.29 x 10-2 ?2 ± 28.21 s.d. at 400x and 41.89 10-2 ?2 ± 6.41 s.d. at 1000x) canine NHL. The correlation analysis between MVD and EA in global canine NHL series was significant at 400x and 1000x (r=0.74; p=0.002; and r=0.73; p=0.002 respectively).
Discussion
Several reports have been published showing that angiogenesis is an important pathway in the development and progression of human NHL. However, to our knowledge no data have been published regarding the correlation between angiogenic index and the degree of malignancy of canine NHL. In the present study we found that MVD and EA significantly correlate with each other and that both paralleled with the degree of malignancy of canine NHL. Similarly to human NHL, increased angiogenesis might play a crucial role in the transition from low-grade to high-grade canine NHL and could explain the biological aggressiveness and metastatic capacity already demonstrated for high-grade canine NHL. On these data we suggest that canine NHL could represent an interesting spontaneous tumor model to study the angiogenesis as an interspecies pathway of tumoral malignancy and biological aggressiveness.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No: 43 • 2005
INDEXED IN EXCERPTA MEDICA

INTERSTITIAL MAGNETIC RESONANCE LYMPHOGRAPHY WITH GADOBUTROL IN RABBITS AND COMPARISON OF FINDINGS WITH DIRECT LYMPHOGRAPHY

Evangelos P. Dimakakos1, Andreas P. Koureas1,Vassilios T. Skiadas1,Georgia Kostapanajiotou2, Athanasios D. Gouliamos1,Lampros J. Vlachos1

1Dept. of Radiology, Areteio University Hospital, Athens,Greece.
2Dept. of AnaesthesiologyAreteio University Hospital, Athens,Greece
Email: edimakakos@yahoo.gr

Key words: Interstitial Magnetic Resonance, Lymphography,Gadobutrol, Animals

INTRODUCTION: The knowledge of the status of lymphatic vessels and nodes often determines probable grave implications, the prognosis and the choice of therapy. For this purpose many different agents have been used to depict the lymphatic system with either positive or negative results and with many or few implications.In this study the aim is to evaluate gadobutrol (Gadovist 1ml/lt) as a contrast agent for interstitial magnetic resonance (MR) lymphography in rabbits and the findings to be compare with direct lymphography.
MATERIALS AND METHODS: Used 12 New Zealand white rabbits, which were injected subcutaneously with 0.5ml undiluted gadobutrol into the hide-leg (n: 9) and the foreleg (n:3), bilaterally. A slight massage was performed at the injection site, immediately after administration, in 9 rabbits. Magnetic resonance was performed in all the rabbits and images were obtained before injection and at 5, 15, 30, 60 and 120 minutes after injection. . Direct lymphography was performed in three rabbits with the administration of lipiodol directly into a peripheral lymphatic vessel.
RESULTS: The images obtained after the direct lymphography and the MR lymphography were almost identical. After the subcutaneous administration into the hide-legs four successive lymph node groups were depicted in 30-45 minutes whereas in the forelegs, four successive lymph node groups were depicted (including anterior and posterior axillary, anterior thoracic, parasternal and mediastinal) with maximum enhancement 5-30 minutes after injenction. The rabbits with massage at the injection site revealed statistical significantly more enhancement of contrast agent in the lymph nodes in comparison with rabbits without massage($P<0.05$). In contrast, comparison of the enhancement profiles of massaged animals receiving with hind-leg(n:6) and foreleg (n:3) injections no statistically significant difference was observed.
CONCLUSION: Gadobutrol is a good contrast agent with favorable safety features which allows the dipiction of lymphatic system within a few minutes after the interstitial administration with the performance of T1 weighted MR lymphography. However, further studies are required to determine the optimum imaging dose and its value in imaging of human lymph nodes with MR lymphograpghy.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2006
INDEXED IN EXCERPTA MEDICA

LACK OF EVIDENCE OF LYMPHANGIOGENESIS IN CANINE AND BOVINE ISLET BETA CELL CARCINOMAS

Elvio Lepri, Monica Sforna, Giovanni Ricci, Silvia Capuccini, Giovanni Vitellozzi

Key words: islet beta cell carcinoma, bovine, canine, lymphangiogenesis, metastases

INTRODUCTION. Islet beta cell carcinomas are uncommon tumors in animals. Despite both normal and neoplastic endocrine tissue is very rich in haematic vascular supply, lymphatic metastases to regional lymph nodes (duodenal, hepatic and mesenteric) are more frequent than blood born hepatic ones. This fact highlight the role of vascular lymphatic system in metastatic spread of beta cell carcinomas. One of the most exciting animal model for the study of tumor lymphangiogenesis is the double transgenic mice ripVEGF-C/RIPtag2, in which VEGF-C-induced lymphangiogenesis rendered locally invasive islet cell tumors lymphangioinvasive and metastatic (Mandriota et al., 2001). The hypothesis is that metastatic behavior is enhanced by extensive intratumoral lymphatic neoangiogenesis, as in human melanomas and squamous cell carcinomas.
The aim of this work is to investigate presence of preformed and newly formed lymphatic vessels in canine and bovine islet beta cell carcinomas.
MATERIALS AND METHODS. 30 islet beta cell carcinomas (25 from cattle and 5 from dogs) have been retrospectively evaluated by light and electron microscopy.
Immunohistochemical evaluation of lymphatic vessels has been performed using D2-40 antibody and antibodies against LYVE-1, Podoplanin, Prox-1, Laminin and VEGFR-3.
RESULTS. In all the bovine cases examined, and in the three canine tumors in which a complete necropsy was performed, there was metastatic spread to regional lymph nodes and liver; in 4 bovine cases metastases were present exclusively in lymph nodes. Histologically the tumors were characterized by well developed intratumoral vascularization, with vascular lacunae, and a peritumoral network of thin walled capillaries, in some of which neoplastic emboli were observed.
Prox-1 and Podoplanin, did not react at all with the tested tissues. LYVE-1 staining was faint and incostant, while D2-40 was non specific, marking also haematic vessels, identified by their erythrocyte content. VEGFR-3 was non reactive in bovine tumors but stained some canine specimens. Double labelling with VEGFR-3 and Laminin did not allow definitive distinction between lymphatic and haematic vessels in canine cases.
Electron microscopy revealed thin vessels around and inside the tumor mass, but was inconclusive in differentiating between lymphatic and haematic vessels, as none of the features typical of lymphatic endothelium (i.e. absent basal membrane, cell fenestrations) were observed.
DISCUSSION. Several markers for lymphatic endothelium have been identified in human medicine Unfortunately none of these have been definitively tested in animal species, and reports are anecdotic. Based on the results of this study, none of the antibody tested is a reliable tool to identify both preformed and newly formed lymphatic vessels in formalin-fixed paraffin-embedded canine and bovine tissues. This could be due to species specific differences, lack of cross-reactivity with human antibodies, or depend on the processing techniques, that have not been standardized for retrospective archive material. Thus the role of lymphatic system in the metastatic spread of canine and bovine islet beta cell carcinomas can't be assessed, even if the evidence of neoplastic emboli in thin-walled erythrocyte-free peritumoral vessels, and the presence of constant nodal metastases, is strongly suggestive of a lymphatic dissemination that can involve peritumoral preformed capillaries as well as intratumoral newly formed vessels. Further studies are needed to confirm intratumoral lymphangiogenesis in animal pancreatic endocrine tumors, as recently confirmed in humans (Sipos et al, 2004).
References: Mandriota et al., EMBO Journal, 2001,20:672-682 ; Sipos et al., Am J Pathol, 2004,165:1187-1197.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

ENDOTHELIN-CONVERTING ENZYME-1 (ECE-1) EXPRESSION AND LYMPHATIC VESSELS DISTRIBUTION IN HUMAN COLORECTAL CANCER

L. Massai, M. Aglianò#, M. Muscettola**, P. Lorenzoni#, A. Pecorelli**, S. Civitelli§, G. Tanzini§, G. Grasso**

**Sezione di Anatomia ed Istologia (Dip. di Scienze Biomediche) Via Aldo Moro 2, 53100 Siena. #Sezione di Medicina Molecolare (Dip. Neuroscienze). §Sezione di Clinica Chirurgica e Terapia Chirurgica (Dip. di Chirurgia). **Dip. di Fisiologia.*

Key words: Colorectal cancer; lymphatic vessels; endothelin-converting enzyme-1 (ECE-1)

INTRODUCTION

The endothelin-converting enzyme-1 (ECE), a metalloprotease with two disulfide-linked subunits (Turner and Tanzawa, 1997), is the main enzyme responsible for ET-1 biosynthesis. It converts the inactive intermediate-form bigET-1 of 38 aa to the mature peptide by cleaving the C-terminal 17 a.a. There is increasing evidence that ET-1 may play a role in the development of colorectal cancer (CRC) both locally and systemically through its mitogenic and angiogenic activity (Ali et al., 2000; Egidy et al., 2000). ECE-1 protein and mRNA was detected in the endothelial, epithelial and glandular cells of various organs (Korth et al., 1999). Immunoreactivity for ET-1 was observed in the endothelium of absorbing lymphatic vessels. ET-1 plays a role in the mechanisms regulating the vascular contractile activity, promoting the lymph flow (Doboszynska et al., 2001). Recent evidence suggests the importance of the presence of lymphatic vessels in CRC (Fogt et al., 2004). The aim of this study was to correlate the presence of lymphatic vessels with the expression of ECE-1 in human colorectal cancer.

MATERIALS AND METHODS

Colorectal cancer specimens were fixed in Bouin's solution and embedded in paraffin. The sections were stained with H&E for histopathological examination and incubated with specific antiserum to ECE-1 and with D2-40, a novel monoclonal lymphatic antibody, for the single and double immunohistochemical techniques. The EnVision peroxidase/alkaline phosphatase detection system or Zymed immunoglobulins alkaline phosphatase conjugated were used. The reaction was visualized with 3-amino-9-ethylcarbazole (AEC)/Fast Red as substrates. Negative controls were carried out omitting primary or secondary antibodies.

RESULTS

In normal tissue, lymphatic vessels were not localized in the lamina propria but just beneath the muscularis mucosae. Instead, several lymphatic vessels were present inside the tumor as revealed by D2-40 monoclonal antibody. By single and double immunohistochemistry, the expression for ECE-1 was localized in the lumen of lymphatic vessels and in the endothelium. In peritumoral inflammatory infiltrate the reaction for ECE-1 was also evident.

CONCLUSIONS

Our preliminary data provide an evidence of possible correlation between ET-1 local production and tumor formation and progression via lymphatic vessels. Furthermore, ECE-1 has been the focus of intense research. In fact, the ECE-1 inhibitors and endothelin receptor antagonists are indicated as potential anticancer agents. Thus knowledge of ECE-1 localization and ET receptors distribution is crucial for effective drug design and delivery. Finally, preoperative plasma bigET-1 levels and the analysis of cellular and molecular expression of ECE-1 and ET-1 might be useful as prognostic indicators in patients with colorectal cancer.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp Co No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

METASTATIC POTENTIAL OF A NEWLY ESTABLISHED TRANSPLANTABLE RAT MAMMARY TUMOR CELL LINE

Thomas Nisslein, Carsten Gehrke and Johannes Freudenstein
Schaper & Bruemmer Co., Medical Development - Veterinary Medicine, Salzgitter, Germany
e-mail:Thomas.Nisslein@Schaper-Bruemmer.de

Key words: rat mammary cancer, cell line, animal model

In many animal models of human mammary cancer, primary tumors show no tendency to metastasize or suffer from other considerable drawbacks. We therefore exstirpated from a female DA/Han rat an estrogen-receptor-positive mammary tumor, which had been induced by a single intragastric dose of 20 mg dimethyl-benz(a)anthracene. We further processed the tumor, thus resulting in a single cell suspension which was then, at a cell count of 1 x 10E6 cells/animal, subjected to three subsequent in vivo passages in female, syngeneic, adult animals. Parallel in vitro cultures were performed in phenol-red free special mammary epithelial cell growth medium designed to suppress fibroblast growth. After varying cultivation periods, when the cells had reached a uniform appearance, 1 x 10E6 cells/animal were transferred onto recipients as well. Starting at 12 days after subcutaneous inoculation, tumors became palpable. In intact female animals, tumors grew steadily over a period of up to 28 days. After this observation period, tumor sizes frequently necessitated that animals had to be killed for ethical reasons.
As soon as subcutaneous transplantation had reproducibly been performed from in vitro cultured cells, we tested in a follow-up experiment for metastatic potential of the cells by injecting either 1x10E4 or 1x10E5 cells/animal intravenously into groups of 5 female DA/Han rats each. 28 days after intravenous injection of 1 x 10E5 cells, metastases of 3-5 mm diameter were found in lungs and regional lymph nodes of experimental animals.
When administered to OVX vs. NOVX animals, it became obvious, that tumors grew considerably retarded in OVX animals. Reduced tumor growth under OVX conditions, compared to intact animals, was evident in primary tumors as well as in the rate of formation of lung metastasis.
Cells were characterized from in vitro culture and in vivo ectopic tumors. Whole RNA was extracted and RT-PCR was performed according to standard procedures using commercially available kits and published primer sequences. With RT-PCR we tested primary tumor specimen for the presence of mRNA for androgen receptor (AR), estrogen receptor (ER) and cyclin D1 (CyD1) as proliferation marker. Interestingly, only mRNA for AR and CyD1 could be detected.
With the here presented cell line, designated 03/664, we have a tool for investigating influences on the metastatic potential of a hormone responsive mammary tumor. Absence of ER mRNA in the primary tumor, isolated after in vivo passages, would correspond to human breast cancer after several cycles of anti-estrogenic therapy. There, tumors often become refractant to the anti-hormonal drug and show an ER-negative phenotype. Further characterization, both in vitro and in vivo, is currently performed, in order to test how various growth parameters of 03/664 cells react to standard and experimental anti-neoplastic drugs.

THE EUROPEAN JOURNAL
OF
lymphology
and related problems
VOLUME 15 • Sp.Co.No. 43 • 2005
INDEXED IN EXCERPTA MEDICA

SURGERY IN TREATMENT OF LYMPHEDEMA; A NEW SUCCESSFUL METHOD

Seyed Reza Moussavi M.D,PhD,FACA Accociated professor of surgery,
Shohada Medical Center, Shahid Beheshti University of medical science , Tajrish, Tehran, IRAN

Key words: Lymphedema _ Modified Homan

Objective :to review common treatment of lymphedema and introduce a new surgical method .Setting :university hospital .participants: patients admitted to vascular surgery department of shohada hospital with diagnosis of lymphedema.

Method: this study was conducted on 96 patient who had referred to shohada medical center with swelling and heaviness of lower extremity as their chief complaint between 1990 -2003 .Doppler sonography was performed before hospitalization for all patients .They were admitted as surgery candidate after confirmation of the clinical appearance as lymphedema and getting assurance about their deep venous system .

Results :All the patients were operated with the modern surgery method (modified Homan).the outcome was excellent with %88 of the patients being completely cured .no complication was detected in their follow up .

Conclusion: According to the difficulties with treatment of lymphedema and common complications and limitations in no treated patients. The new method can be considered as an appropriate alternative way, but also might be accepted as a standard surgical method.