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PANEL DISCUSSION: DIFFERENCES

DR. M. WITTE: Let's begin with the following questions:

"Are there any things that blood capillaries have that lymphatics don't?"

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"Are there things that one has in such great excess, or such scarcity, that they might be distinctive?"

DR. LEAK: I could start off by saying that for the lymphatics for the most part, the endothelial cells have many more microfilaments. Many of these have been shown to be actin filaments by cytochemical as well as biochemical means in contrast to the normal blood capillary endothelium. These microfilaments have been shown to increase in certain disease states but normally, for the most part, especially the muscular capillary endothelial cells don't have as many.

DR. CASTENHOLZ: Many investigators insist on the statement that initial lymphatic capillaries don't have a complete basal membrane. I think it's only a question of definition. If you use the classical definition according to which the basement membrane contains ground substance and reticular fiber system, you have to apply this term also to initial lymphatics.

DR. LEAK: You might recall in our initial descriptions of initial or lymphatic capillaries, we differentiated between basal lamina and basement membrane. The basal lmaina is the very narrow band that is continuous around blood capillaries, which has recently been shown to contain many collagenous proteins. For the various lymphatic capillaries, you don't have a continuous basal lamina. You start to get a very well defined basal lamina when you get into collecting vessels, and this becomes continuous as you go up to the larger vessels where you have smooth muscle cells. So, I would like to make a distinction between basement membrane in the classical sense of the word, basement membrane being the ground substance which you don't see at the light microscopic level under most cases.

DR. RYAN: I have a question for Dr. Leak. I'm interested in the comment you made about cytoskeleton in lymphatics. I've often wondered whether this was associated with the important spreading and lining function of the lymphatic endothelium. It could line tissue spaces. As it spreads itself very widely, I have tried to use this particular feature with one of my colleagues to recognize lymphatic endothelium in cell culture. When one looks at endothelial cell culture, say from the skin, one finds a whole variety of cells. One tends to say that anything which isn't stained with Factor VIII-related antigen or other specific stains, then it is not endothelium. One rules it out. But one is still left sometimes with these very large widespread cells with small nuclei and then wonders whether those are in fact lymphatic endothelium.

DR. LEAK: With many cultures of epithelial cells now as well as blood vascular endothelial cells, a lot of investigators are using markers for actin, which show very nicely the increase in polymerized actin filaments in cell culture in contrast to *in vivo* conditions.

DR. M. WITTE: But, that would not be a point of distinction because the actin filaments clearly, we think, are present in lymphatic endothelium. I think Miles Johnston actually can speak to this, and he's nodding to me. I can just summarize his own observations in bovine lymphatic endothelium from the mesenteric duct in which he found approximately half of the cells were positive for Factor VIII and the other half weren't, which I think is germaine to Dr. Ryan's point.

DR. CASLEY-SMITH: All we've been mentioning really have been quantitative differences. As far as I can see, apart from Dr. Ohkuma's poster and apart from fenestrae, there are no absolute differences between blood vessels and lymphatics. I'm not too sure whether your enzymes and your histochemical work is qualitative or just quantitative, but none of the rest I've heard of so far, apart from fenestrae, is distinctive. It's the same cell. Just one spreads a bit in one direction; the other spreads a bit in the other direction.

DR. M. WITTE: And Dr. Ohkuma, you were actually bringing up that when you have a lymphangioma, all bets are off in terms of any definition. Perhaps you want to respond to Dr. Casley-Smith.

DR. OHKUMA: The difference between lymphatics and blood vessels is that one contains lymph, the other contains blood.

DR. M. WITTE: And sometimes lymph is bloody and sometimes plasma skims.

DR. OHKUMA: Their functions are so similar, and even my enzyme studies are largely quantitative not qualitative. It's very difficult to differentiate.

DR. O'MORCHOE: I would make the point

though that even though few if any absolute differences between blood and lymphatic endothelium have thus far been documented, we're going to find increasing numbers of mild differences--differences in function from a chemical point of view, from the electrical point of view, and various other points of view. This is because the function of lymphatic endothelium is grossly different from blood capillary endothelium. The difference is great. The blood capillary endothelium is designed to keep protein in, to a large extent, and to control the permeability outwards (i.e., the substances that traverse the endothelium). I think the lymphatic endothelium, on the other hand, is designed largely to transport substances in the opposite direction. The two are very different and I think that we're increasingly going to see these differences. Histochemistry is a very gross way of defining the differences between these two endothelia and with more sophisticated molecular probes, we should see major differences between these two vessels--two different types of cells.

DR. C. WITTE: I wanted to ask Dr. O'Morchoe, did those vesicles that stained with albumin, or those other particles, did they ever reach the abluminal surface? In other words, when Dr. Simionescu showed his slides, I only saw the albumin and the vesicles filling near the luminal surface, but do they ever get to the other side? Are you suggesting, in effect, that vesicular transport is the way albumin is being transported in the kidney?

DR. O'MORCHOE: I think that if you take the *in vitro* system that we used, which granted is open to some criticism as are all *in vitro* systems, we simply had a lymphatic vessel cannulated at both ends and perfused in a bath and added horseradish peroxidase to the bathing fluid. You then can follow this substance at the various stages across the lymphatic endothelium from the interstitial or abluminal surface to the luminal surface and luminal caveoli. It seems that the horseradish peroxidase is in greater concentration; that is, it's denser along the luminal surface and then the luminal caveoli. On the other hand, if you add it to the perfusion fluid, it does not pass across to the other side, which one would expect if it travels simply in a concentration gradient. Regarding one other critical point about transport through the vesicular system, the questions have been raised, "Are there in fact vesicles; do vesicles form; do they shuttle or move across form one surface to the other; do they move backwards and forwards?" There has been substantial evidence to show that at any one time most of the vesicles communicate with one or other surface. Using tannic acid on lymphatic endothelium, we showed that about 80-90% of the vesicles were in communication with either the luminal or the abluminal surface at any one time and that they interconnect. I think that the idea of a vesicle forming and running across the cell and opening on the other is a bit naive and simplistic. More likely, these interconnecting vesicles lose their connection with one surface and develop a connection with another, and there may well be membrane movement. Dr. Simionescu was asked that question about the movement of vesicles and he didn't seem to want to answer it. My answer is that it's simplistic to think of them running across, but there is definitely evidence for a sort of movement of membrane.

DR. M. WITTE: I have just a rhetorical question for Dr. O'Morchoe to consider for a few minutes regarding the structure-function relationships of the two systems. Are the sinusoids of the liver and the sinusoids of the spleen designed to keep out protein and particles, or are they in fact perhaps designed for quite the reverse? What happens, as Dr. Ryan has pointed out, when the lymphatics are not able to do their job? Is the blood vascular system designed in such a way that it can't respond?

DR. RYAN: I was worried yesterday by Dr. Simionescu's beautiful lecture and in the same way by Dr. O'Morchoe's concepts. It seems to me that there's just too fine a control to rely on anionic sites or cationic sites in microdomains. You've only got to have a little bit of injury or inflammation, and the system breaks down. The lymphatics are a sort of safety valve system; when permeability factors are not working too well it shouldn't be necessary to rely on something so finely selective as that mechanism, useful for directing things in healthy tissues but breaking down when a little disease supervenes.

DR. M. WITTE: Is the kidney perhaps different from the liver in what it's intended to do normally and abnormally? Can we leave you with that rhetorical question for a couple of questions?

DR. CASLEY-SMITH: I also want to have another go at Professor O'Morchoe. The kidney, liver, and thyroid, where you find no big open junctions, are all situations where there is a positive tissue pressure in a tightly encapsulated organ. I have suggested that it is quite conceivable that initial lymphatics function in two modes. In some places, such as the skin and the gut, they pump and leave junctions open and shut. In other areas where there is a positive tissue pressure, the junctions are as you can see quite narrow, about 20nm, but that is more than enough to account for the amount of lymph flowing out of those vessels. But it's not a pumping action any more; the lymphatics are simply acting as conduits and the body uses the same structure for a rather different way of functioning.

DR. CASTENHOLZ: I should point out the great differences between initial lymphatics and blood capillaries as regards endothelial organization and structure differentiation. If you look at our findings on scanning electron microscopy, there are some peculiar phenomena. One is a flat area of endothelium composed of flat broad cells bordered by wavy lines, not described in blood capillaries. The other phenomenon is in some regions of the endothelium, prominent branched cells occur which may function to control the open junction. There is also a special structural organization of the outer vascular

wall, a reticular fiber system. I have a question to Dr. Ryan. We have similar findings to what you showed in one of your slides by reticular fiber staining technique. This demonstration resembles what you have shown with your elastic fiber staining. Would you agree that there are two systems? One is the elastic fiber system, and the other is the reticular fiber system concentrating around the lymphatics, which are intermingled?

DR. RYAN: I would certainly agree. My main question is could the large elastin fiber, such as Professor Hauck described in the mesentery, be a preferential channel traversing the matrix? I agree that both types of fibers may be attached to lymphatics as shown by Dr. Leak amongst others, but the question is, are they preferential channels and fibers? I don't think collagen is a preferential channel but I suspect that elastin might be.

DR. MAGARI: In relation to Dr. Casley-Smith's talk, I would like to hear from Dr. Leak on the problem of the interrelationship between elastic fiber and anchoring filament.

DR. LEAK: In most cases, especially in the dermis, we find that the anchoring filaments extend. You can see them go for quite some distance out into the surrounding tissue between both elastic fibers and collagen bundles. We also find when we fix by perfusion and use chemicals such as acrolein to preserve many of the filaments, we can see small bundles of elastic fibers very close to the smaller lymphatic capillaries. So they are there, and we did some preliminary studies once to see if we could digest elastic fibers and then stain the beginning portions and the capillaries. They are very close to the anchoring filaments and the wall. But I've not convinced myself that we were able to remove these elastins so we still have not determined what the chemical make-up of these are.

DR. MORRIS: Could I ask two questions both with immunological connotations to them. The first one is to Dr. Witte, who during his presentation talked about the role of the lymphatic system in immunosurveillance. It seems implicit in that proposition that there is some analogy between acquired tolerance and the natural tolerance that enables self-, non-self-discrimination to occur quite early on and prevents the body's immune system from destroying itself. It always seems to me that one of the functions of lymph nodes in regards to cells is to ensure that the metastatic behavior of lymphoid cells and other cells is promoted. In other words, lymph nodes play a special role in ensuring the distribution of cells around the body. I do not believe there is any evidence whatsoever for equating acquired tolerance with natural or self-tolerance. I wondered if he would like to make some comments on the possible role that lymph nodes play in ensuring that metastatic tumor cells are distributed as widely as possible throughout the body. My feeling is, of course, that we need a new explanation as to why tumor metastases occur so widely in the body and that almost certainly relates to the proposition that those cells have lost the capacity to "home" back to the original tumor whereas lymphocytes have that capability to a very highly developed extent. The other proposition is to Dr. Ryan because he mentioned that in some of the so-called preferred immunological sites, functions of the lymphatics were taken over by blood vessels. I would like him to comment as to why you are still able to maintain a preferred immunological site when you've got vessels modified to in fact absorb antigenic materials.

DR. M. WITTE: I think the answer to those questions would probably win at least two Nobel prizes.

DR. C. WITTE: Needless to say, she stole my thunder already. I think Dr. Morris could probably answer that better than anyone. The best I can do is recognize that on the one hand, bacteria will be filtered by nodes. There'll be a process that seems to destroy them or at least entrap them. Immunologically phagocytic processes then take place that digest and eradicate the problem. This phenomenon may also occur in malignancies. Indeed, for a long time, it was thought the best thing to do was to remove regional lymph nodes on a clinical basis. Then it was decided that the best thing is to leave them behind. The issue then arose, do metastases occur from lymph nodes themselves? In short, I don't think the answer is known except that on the one hand, lymph nodes seem to protect; on the other, they are clearly a major focal point for metastases to grow and proliferate. Alternatively, they may be the only clinical manifestation at times of an underlying tumor somewhere. So, at the moment, I don't know whether those germinal centers and adjacent lymphocytes are circulating around again, or whether they're statically there simply doing their thing. I would say that's a major issue in malignancies in general, and I'm overwhelmed by the challenge to solve this problem.

DR. RYAN: I'm impressed that in order to develop good cellular immunity, it is a good thing if materials go through lymph nodes. You get T-cell stimulation this way. I'm also impressed by some data which suggest that when antigen gets overwhelmingly into the blood vascular system without going through the lymph nodes, you get T-cell suppression. This has been shown by the mycobacterial antigen. It's also been shown with various other materials, that if it goes directly into the bloodstream and doesn't go through the lymph node, then you get T-cell suppression. Therefore, I wondered if it is possible that in the skin sometimes materials get directly into the blood vascular system and don't go to the lymphatic system. This is still an open question. I then asked, what would be the morphological feature of skin if antigenic material is getting directly into the blood vascular system and not into the lymphatics? One might see dilated and dysfunctional lymphatics with changes in vascular endothelium similar to what's seen in post-capillary venules of lymph nodes or lymphatio-venous shunts,

both of which might allow transport of bulk protein and antigen directly into a vascular system bypassing the lymphatic system. I'm really posing a question without answers, but it's important to explore.

DR. C. WITTE: On the other hand, Dr. Ryan, you do have some expansive organ systems in the bloodstream which continuously accommodate loads which bypass the lymphatic system, for example, the spleen, the bone marrow, or the thymus. Perhaps the subtleties of taking out the spleen, or for that matter the appendix, are yet to be revealed in that regard.

DR. RYAN: We did some experiments with carbon and also with Leishmania some time ago where we injected these agents into the animal's vascular system. Nothing happened unless you first paralyzed the Kupffer cells and the splenic RES, which have a tremendous capacity for trapping. After paralysis, you found all sorts of amateur macrophages picking up these particles, and you began to get a situation like leprosy. Carbon and Leishmania began to pour out of the nose into serous fluid, something which wouldn't happen unless you first paralyzed or overloaded the liver and the spleen.

DR. MORRIS: Can I just destroy the theory that you need to have peripheral sensitization to get good cellular immunity? One of the most vigorous of all reactions, of course, is the destruction of the kidney allograft and that can and does take place by sensitization of the host, the host cells within the vascular compartment. The regional lymphatics are not involved in it at all.

DR. M. WITTE: I think we've seen over the past few minutes a sample of what can happen when morphologic lymphologists get together to talk about structure. We started with a closed junction and we're now talking about Leishmania infection and kidney transplantation. We will, of course, return in the next two discussion periods to these issues. I wonder if I might ask a question that relates to the opening address by Dr. Zwefach, in the Beijing meeting, where he drew a picture of the microcirculatory unit. I was surprised that he had a "unit" with artery and vein cascades and branches without a lymphatic in there draining somewhere.

DR. OLSZEWSKI: We're talking mostly about the structure of the small lymphatics, but not so much about their function. May I ask the panelists whether the endothelium of initial lymphatics has any phagocytic properties or if it's not phagocytosis just engulfment of particulate matter. When we inject clinically non-pathogenical Streptococci in experimental animals, we often see the bacteria stuck to the wall of the endothelial cells as well as extravasated through the wall of capillaries or even bigger lymphatic vessels. Has anybody documented phagocytosis of bacteria by the lymphatic endothelium because this might be one of the basic differences between the lymphatic and blood vessel? So that would be the first question, then I have another one. Is there any difference in the proliferation of lymphatic and blood capillaries, for instance, in the wound and could one differentiate between the very small vessels sprouting into the wound? That would have a tremendous clinical implication just for understanding the wound healing process, healing in skin grafts, transplants, etc.

DR. M. WITTE: Could we have a couple of morphologists answer? I think that really is a very important clinical question.

DR. CASLEY-SMITH: If I could answer his second question first. It's well known that the initial lymphatics grow in more slowly than blood vessels during wound healing.

DR. OLSZEWSKI: But how can they be differentiated? This is just the point of my question.

DR. CASLEY-SMITH: Well, this has been done in ear chambers where the two have been differentiated after the event by following the vessels and some days later you can tell which is which. Regarding your earlier question on phagocytosis. In 1964, I observed that initial lymphatic endothelium will take up carbon into very large vacuoles. In fact, if you overload the endothelium, the cells migrate out, lie in the tissue and they look just like macrophages, but they're originally endothelial cells. As far as I know, no one has ever looked at bacteria.

DR. RYAN: But they have in vascular endothelium. In lepromatous leprosy, vascular endothelium becomes extremely phagocytic and is full of bacteria, so I don't think it's a distinguishing point.

DR. M. WITTE: Hepatic sinusoidal endothelum in tissue culture will take up latex particles and can become intensely phagocytic when Kupffer cell function is depressed, the endothelium itself begins to take on the function of the Kupffer cells.

DR. CASTENHOLZ: We have done experiments with interstitial injections of carbon into tissues. We saw the initial lymphatics surrounded by a halo of carbon particles. Our findings correspond to those of Professor Casley-Smith where carbon particles were engulfed into the initial lymphatic endothelium and not into the endothelium of blood capillaries.

DR. LEAK: Just a follow-up on that. We did some long-term studies with injection of latex spheres about a micron in diameter as well as carbon. Up to 12 months you can see extremely large vacuoles in the lymphatic endothelial cells that have taken up the carbon and still retain it because they can't digest it. It just stays there until the cells die.

DR. O'MORCHOE: Let me just comment and come back to the point John mentioned. Is the kidney, is the liver, is the thyroid different from elsewhere? The answer is probably "yes", and again to your question about the endothelium of the liver sinusoid, is that different? I would say "yes". I think that we will be able to pinpoint those differences as we develop molecular probes and chemical probes much more accurately than we can do now. The sinusoids of the liver are designed, as you say, to allow protein to move in a different direction, but let me

come back for a moment to the kidney and liver. The mechanism of lymph formation is almost certainly different, and one can see that just by taking an anesthesized animal such as a dog and cannulating the thoracic duct. The bulk of the lymph flow in the thoracic duct is coming from viscera like the kidneys and the liver. Together they account for most of thoracic duct flow.

DR. M. WITTE: I thought you were going to end on an uncontroversial note. We would disagree with your last statement. The bulk, at least 75%, of thoracic duct lymph clearly comes from the intestine.

DR. O'MORCHOE: That depends on when the animal was last fed and on the activity of the gastrointestinal tract.