Lymphology 21 (1988) 70-80

PANEL DISCUSSION: PATHOGENESIS OF AIDS AND KAPOSI'S SARCOMA

DR. ZIEGLER: There are two large issues to address this afternoon. One of them is the pathogenesis of AIDS and the other is the pathogenesis of Kaposi's sarcoma which I think we will agree is some form of epiphenomenon--a side show if you will--but one that has intrigued everybody who has looked at the problem because of the multiplicity of other factors that seem to confound this neoplasm. But before we get to Kaposi's sarcoma maybe we will address a few of the problems that have come up in the pathogenesis of AIDS. Let's start with the pathologists who've made some very important observations and see if we can reach some agreement. It sounds to me like the progression of disease moves from a proliferative state into an atrophic state, and we must assume that the virus itself has something to do with putting this sequence of events into motion. Would anybody like to address just that first phase of pathogenesis of AIDS? Why do these lymphoid cells suddenly become so hypertrophic and hyperplastic?

DR. RACZ: The light microscopic alteration shows changes in the follicular and germinal center typical of viral infection or immunologic stimulation. Within the germinal centers you can discriminate between two areas. In one area, the so-called densely populated area, there are blast cells we call centroblasts. There are the tingible body macrophages and there is the so-called thinly populated area of the germinal center facing usually into the marginal sinus in the direction where the antigens are coming. In these areas, the dendritic cells are very active, more active than in the densely populated area. This is where the T4 CD4 lymphocytes are located. If you have an acute infection, then the ratio between these two areas changes and the so-called densely populated area with many centroblasts and tingible body macrophages extends and descends making the populated area thinner. This is what we think goes on in the first phase. This is not specific for HIV infection but the extent is distinctive. There are diseases in which you can see similar but not such extensive hyperplasia of the follicles. Dr. Tenner-Racz has carried out the immunohistochemical and electron microscopic studies.

DR. ZIEGLER: Let me just interrupt and ask if from the slides I saw there were really only one or two cells that stained with the *in situ* reagents? Do you think that the follicular hyperplasia of that entire lymph node is based on just a few infected dendritic cells or are we just not picking up all of the antigenic material?

DR. RACZ: This is a main point we would like to emphasize, that the number of infected cells is not just a few. If we perform serial section, we find that these are not isolated cells. Second, on an electron microscopic picture, such as Klara has shown, you see in such a small area 6 or 7 viral particles. The infected cells are not as few as you think. Using monoclonal antibodies against the core proteins, the number of infected cells in the germinal centers is much greater. One important point is that in contrast to the blood where the number of the viral particles is low, in the organs, in the lymph nodes, in the germinal center, probably in the brain and the skin, there are many more viral particles. So, if you ask us about the reason for the extensive hyperplasia, then the load of viruses has to play a role.

DR. ZIEGLER: That's a good point to make experimentally.

DR. TENNER-RACZ: If you perform immunohistochemistry with monoclonals against core proteins, it is possible to see much deeper or heavier deposition than you expect on the basis of electron microscopic findings. So we speak always about intact virions that you can see in the electronmicrographs. But it is possible that in the germinal centers you can find viruses that degenerate. So part of the deposition you detect in immunohistochemistry could represent degraded viruses. But if you take into consideration that *in vitro* you can activate B cells even with viral proteins from killed virus, deposition of viruses or viral proteins in the germinal centers on the surface of antigen dendritic cells is probably responsible for the histology of the lymph node and the B cell activation. Just one other point. The number of FDC in the germinal center is probably not very high. In animal experiments, in lymph nodes of mice immunized with sheep red blood cells, the FDC account for approximately 2% of the cell populations but their surface density is ten times higher than that of the lymphoid cells. So, this huge surface area expressing CD4 antigen can be very important in the pathogenesis of AIDS.

DR. DIEBOLD: I completely agree. We must stress that follicular dendritic cells are immune accessory cells in all immune reactions whereas B lymphoid cells are concerned with humoral immunity. Antigens which are injected in experimental animals, for example, arrive in the lymph node through the lymph and are concentrated in the serous space between the cytoplasmic processes of the follicular dendritic cells. These latter cells express receptor for the Fc

part of the IgG and IgA and for three types of complement--C3, C3-inhibitor, and CDG3. Probably in AIDS some of the viruses stick on the follicular dendritic cells and have common immune complexes. So, I think probably in the beginning a B cell hyperplasia takes place as a reaction against HIV. Perhaps the infection of the follicular dendritic cells is secondary to some of the viruses produced by CD4 in the germinal centers or from viruses which circulate in the lymph or in the blood.

DR. ZIEGLER: It has been shown experimentally that *in vitro* three other events that turn on B cells are the Epstein-Barr virus, the cytomegalovirus, and also cytokines from infected T cells. This point has all been shown experimentally but it's very hard to determine. Of course, HIV or the HIV protein does it directly but it's hard to determine which of all these events plus the other things you've mentioned get these little B cells switched on.

DR. M. WITTE: I just would like to interject another total unknown, that is, the microcirculatory aspects of lymph node pathology. How much of what you see and even differential immunoarchitectural changes are due to alterations in blood flow, either total blood flow or the way the blood goes, in terms of which cells are favored or not? I think almost nothing is known about the blood circulation but I would be willing to bet important changes take place just as if you tie off the splenic artery or constrict it, you get changes in splenic cell populations. If you obstruct the lymphatics going in or going out, you get profound changes in the immunoarchitecture and differential survival or reduction in cell populations. During the inflammatory response and infection, all sorts of things are happening and many of these events are microcirculatory phenomena in the truest sense of the word.

DR. ZIEGLER: Is there any evidence from an anatomical and structural point of view that lymph nodes that become so vastly hyperplastic actually reverse lymphoid flow or obstruct it so that it doesn't reach its next destination?

DR. WITTE: I wasn't even thinking in those terms. I would think that late on that might occur.

DR. RYAN: I don't think you need to postulate that. All you have to think about is the lymphaticovenous shunt system developing and that seems to be a vital later consequence which may turn on further immune problems. If one goes back to the old days, researchers studying chrome sensitivity put chrome directly into the bloodstream and produced T cell suppression; then more recently, they did it with mycobacteria. You put the bacteria directly into the bloodstream, then you get T cell suppression and B cell activation, and that is a bad thing for the immune process. It's bad news when you get lymphaticovenous shunts and your information which should be going for the right process with the lymph node is instead shunted either centrally or peripherally and both are happening?

DR. WITTE: In addition, the production of high endothelium must have differential effects on which cells migrate in and which recirculate. I'm just raising it as a big unknown.

DR. ZIEGLER: Since we are trying to focus on ways to actually explore how you might go after these hypotheses, are there experiments that come to mind that would test some of these notions?

DR. DORFMAN: I'm not completely familiar with the work but one of the young assistant professors in our department, Eugene Butcher, is doing some very elegant work on the relationship of lymphocytes to high endothelial cells, that is, migration of lymphocytes through high endothelial cells in the Peyer's patches vs lymph nodes vs tonsil, and so on. This sort of study is a very important one. He's developing monoclonal antibodies to these lymphocyte-high endothelial interactions and actually testing these against different malignant tumors such as Burkitt's and other lymphomas and finding that there are different reactivities of these monoclonal antibodies to different lymphomas.

DR. ZIEGLER: Yes, I think the whole area of lymphocyte and monocyte traffic is going to be one we will learn much more about as monoclonal antibodies are accrued.

DR. RACZ: As I have demonstrated today, a wall of the high endothelial venules in the first phase of the HIV infection was full of lymphocytes. What this means is increased lymphocyte traffic from the blood into the lymphatic parenchyma. But the other very important point is that the high endothelial venules are dynamic structures. This means that the part that is high endothelium is increased and during the infection there is a large increase in the length through which the lymphocytes enter and this is what we see in all the lymph nodes. So there is extensive change in the lymph node during infection.

DR. WITTE: Experimental models that could be and are being looked at to some extent include tying off the afferent lymphatic, tying off the efferent lymphatic with or without venous obstruction, increasing arterial inflow, and just by analogy we have been interested in the changes in splenic function that accompany increased splenic flow. If you raise splenic flow, you will greatly augment the phagocytic function of the spleen and patients will become hypersplenic. If you reduce splenic circulation dramatically, you can cure hypersplenism without taking the spleen out. In other words, organ function is closely linked to blood flow. I only raise the question because it's almost entirely uninvestigated and it's not at all irrelevant to what goes on in infection to the lymphatics, arteries, and veins. The high endothelium is just an additional aspect. The other way some of these phenomena are being studied is *in vitro* with endothelial monolayers and looking at fixation and movement of lymphocytes across the monolayer, in the presence or absence of virus, or viral products, etc. I don't think there's been much studied in this area.

DR. DORFMAN: I wondered if I could raise this point since you are discussing obstructive phenomena and Klara had intended, I believe, to talk about this peculiar phenomenon of so-called venous transformation of lymph node sinuses secondary to venous obstruction. This condition resembles Kaposi's sarcoma but it really isn't the same, is it?

DR. TENNER-RACZ: There are some experiments in animals where the phenomenon has been produced by ligation of the afferent and efferent vessels. And in some cases we observed this feature in HIV-infected patients. It is interesting from the diagnostic point of view not to mistake it for Kaposi's sarcoma but often the resemblance is so great that vascular sinus transformation appears to be a benign variant of the Kaposi's sarcoma.

DR. DORFMAN: It is quite a difficult diagnostic problem. I have seen cases misdiagnosed as Kaposi's sarcoma--cases of so-called vascular transformation.

DR. WITTE: And you're convinced it's different--that it's not possibly part of the same process?

DR. DORFMAN: I'm convinced that it's a reactive process that doesn't lead to the pathological phenomena we associate with Kaposi's sarcoma and should not be so diagnosed.

DR. ZIEGLER: Any final comments on the proliferative phase?

DR. GOTTLIEB: I wanted to raise a point about this activation matter that we've really not settled fully. Let's assume based on the evidence that there are clearly viral antigens on these dendritic cells and they stimulate the group of cells around them. Yet, we have a rather greater degree of B cell hyperfunction as reflected in hypergammaglobulinemia that would seem to be reasonably accounted for by these specific cells triggered by viral antigen. For example, if one follows over time the level of antibody to a variety of viral antigens, my impression is that one doesn't see these levels rise constantly over time. On the other hand, the degree of hypergammaglobulinemia does appear to rise over time until the system basically runs down. I wonder if someone would comment on that phenomenon. The other interesting point we haven't discussed is the issue of the MHC determinants, the DR determinants, and in particular, whether you've done any work to map those on the surface of these antigen-presenting dendritic cells. It is said and the report that I alluded to this morning contains the statement that in HIV infection, as the disease progresses, the MHC determinant is lost, which would suggest that the residual viral antigen is there to provoke the cells but it doesn't have an adequate signal to give a specific antibody response. These are intriguing questions to explore.

DR. ZIEGLER: Let's take them in two parts. Would you just capsulize the first part of your question?

DR. GOTTLIEB: The first question is, how do you account for the profound hypergammaglobulinemia which appears to be generalized and not directed against the virus, if in fact, the supposition is that you're triggering B cell proliferation with viral antigen?

DR. RACZ: Perhaps I can start. If you perform an autopsy in the early stage of the disease, you see hyperplasia of the lymphoid tissue not only in the lymph node but, as Jacques emphasized, in the spleen. Then in the tonsil and in the intestinal tract, there is hyperplasia of the lymphoid tissue in the whole organ, and there are large numbers of B lymphocytes and plasma cells.

DR. ZIEGLER: I was going to just say that there are at least 4 or 5 *in vitro* experiments that can stimulate the B cell lymphocyte to proliferate in this fashion, but we don't know what goes on *in vivo*. I don't know how we can take this particular issue much farther.

DR. RYAN: In all diseases where there is a range of immune suppression, you have this spectrum, as in leprosy or leishmaniasis, where at one end of the spectrum the T cell system is totally suppressed and you've got very high B cell activity. This phenomenon isn't specific to AIDS at all. It is specific to the problem of suppression of the T cell system.

DR. ZIEGLER: Let's address the MHC issue as well.

DR. TENNER-RACZ: I don't say that deposition of viral proteins in the germinal center is the only cause of this hypergammaglobulinemia. But regarding EBV or CMV as the responsible agents, there are well documented cases in the literature without this kind of infection. Just to give an example, we have a heterosexual man who visited Brazil, came back to Germany, and had a mononucleosis-like illness resembling Epstein-Barr infection but the negative serology did not support this diagnosis. The symptoms disappeared and the physicians thought he might have a tropical disease. An HIV serology was done which was negative. Then he converted. A lymph node was removed, and viral deposition was already present in the germinal center. The hypergammaglobulinemia was not due to Epstein-Barr virus or CMV.

DR. ZIEGLER: Does anyone have any data to speak to the question about MHC in the lymph nodes, the major histocompatibility complex? Basically, from my understanding there are discrepant results on the regulation of Class II antigens induced by HIV *in vitro*, which seems to down regulate the expression of Class II antigens. *In vivo*, there are some macrophages that show regulation and the Langerhans cell experiment was an example of that. But, there are other situations in which MHC might be overexpressed. I don't think the answer is in on that at all.

DR. DIEBOLD: On frozen tissue section, there are many cells which express HLA-DR but it is difficult to know what type of cell it is because they are close together. We just don't know which cell expresses HLA DR and which cells lose this expression.

DR. ZIEGLER: The answer to this question is

important because it bears on the hypothesis that I presented, that is, if the virus is somewhat like HLA-DR antigens then the antibodies are going to latch on to the major histocompatibility antigens. Clearly that idea needs to be addressed.

DR. TENNER-RACZ: Our data for dendritic cells can detect HIV antigens. *In situ* it is very difficult.

DR. ZIEGLER: Yes, I would have thought that one experiment might be to isolate them and then add mixed patient serum and see if the serum blocks the specific monoclonal. Blocking experiments are not easy to do and they are fraught with artifact but that's one way to get at that question. Let's move on to the pathogenesis of AIDS, and then what happens to these lymph nodes. Why do they dissolve? Why do they atrophy? What is going on? Jacques, you brought up the question of the CD8 lymphocytes infiltrating as being a unique phenomenon, explicitly distinctive in AIDS, and not seen in other conditions. Can you expand on that? Do you think these cells are destroying the follicles?

DR. DIEBOLD: I cannot give an answer to your question. What we see is when you compare lymph nodes from successive biopsies in the same patient, the two most important findings, and I think Paul and Klara have the same result, is first the follicle lysis. This important process is the beginning of B lymphoid depletion. The second modification in evolution is the decrease of total number of T cells predominantly of $CD4^+$ cells. We almost never see such an increase of $CD8^+$ cells in the germinal centers in other diseases. Even when we see some $CD8^+$ cells in lymph nodes from viral disease, we never see such a great increase and particularly associated with the CD4 decrease. I just don't know exactly the significance of this event.

DR. ZIEGLER: Is it clear whether these are CD8 killer cells vs CD8 suppressor cells or hasn't that been worked out?

DR. DIEBOLD: I cannot give you an answer. Perhaps among the CD4 decreasing cells are some cells that are CD4 inducers of the suppression. So perhaps the decreased number of CD4 is involved in the B hyperplasia but that's just an unproven hypothesis.

DR. RACZ: The most characteristic finding is the decrease in number of CD4 cells. We haven't seen enough lymph nodes from other viral infections because lymph node biopsy isn't often justified in viral disease unless the diagnosis is uncertain. Increased numbers of CD8 cells may be found but not as extensively as in HIV infection. In the early phase of the disease, one sees many pyknotic dying lymphocytes--this is what you see in the follicle and the reason so many tingible body macromolecules are phagocytosing pyknotic lymphocytes. These are signs of lymphocytes dying.

DR. DORFMAN: It's obviously speculation but if the dendritic reticulum cell is so necessary in the activation of B lymphocytic function, infection of the dendritic reticulum cell by virus may initially stimulate follicular hyperplasia but as more and more infection occurs, one can envisage that the function of this cell is such that its interaction with the B lymphocyte suffers. Similarly, the interdigitating reticulum cells are said to have the same interrelationship to T cells in the thymic dependent areas. So, you're having perhaps involvement of both dendritic and interdigitating cells and a lack of normal interplay between these cells, a depletion of lymphocytes, replacement by plasma cells. Where the plasma cells come from I don't know. The B lymphocytes are still functioning reasonably well; at least they're causing hypergammaglobulinemia. The T lymphocytes are functioning inadequately.

DR. ZIEGLER: Any other comments on this question of pathogenesis?

DR. WITTE: Just to reiterate the point about the afferent limb. Granted you're not seeing pyknotic lymphocytes but the end result of atrophy and fibrosis certainly occurs even in non-inflammatory processes. In the congenital lymphedemas that don't have the afferent limb coming in there, the nodes will look very much like the node that is the end result of inflammation and at least that may play some role.

DR. DORFMAN: When you're talking about lysis, do you mean only of cells or is there lysis of the connective tissue supporting system? If so, then agents like collagenase and elastase are being activated, and then obviously the whole protease system is being activated, which is not unusual with transformed cells and has implications for cell surface membranes and all sorts of other things.

DR. DIEBOLD: I think there is no destruction of the connective tissue but you know in activated germinal centers there are very few fibroblasts and collagen.

DR. ZIEGLER: Before we leave this topic, virologists have told us that *in vitro* these cells form syncytia and they balloon up and then they die very quickly in a matter of days. One of the intriguing questions is whether this *in vitro* cytopathology is relevant in the lymph nodes. From my understanding, except for the brain, the finding of syncytial cells, giant cells, is exceedingly rare. Do you think that this might be happening and then these cells vanish before the time you take the lymph node out or do you think this is not a relevant pathogenic mechanism? Ron, why don't you start?

DR. DORFMAN: There have been reports in the literature of cells which have been called polykaryocytes resembling Warthin-Finkeldey giant cells. These can be seen in reactive lymph nodes and lymph nodes involved by lymphoma. Kim and Kelsberg published an electron micrographic study showing that these are multinucleated cells. They seem to form a syncytial mass with multiple nuclei. We described a small percentage of our cases in AIDS patients with these polykaryocytes but we really didn't think they were significant enough. Others have suggested that they were significant, that this was one of the important findings. I wondered if

you had encountered these cells and whether you feel that they are significant?

DR. DIEBOLD: I didn't speak about this because I don't think it's very important for the diagnosis. In my experience in 20% of the cases you can see some so-called Warthin-Finkeldey and this polykaryotic cell can be found either in the germinal centers, interfollicular or the cortical areas. For me these cells are not specific and I agree with you, we can find them in some other reactive lymph nodes, lymphadenopathies, viral diseases. I don't know if they are the same type of cell as found in follicular lymphoma. One hypothesis was that in the follicles, these cells could perhaps represent peculiar follicular dendritic cells because normal dendritic cells are often binucleated and in some infections perhaps or in some reaction, follicular dendritic cells can have this appearance. This is only speculation.

DR. BARRE-SINOUSSI: I just wonder if in fixed cells you will be able to see such giant cells. From the *in vitro* experiment when we tried to fix the cells with dye immediately, it was impossible for us to see these giant cells very easily.

DR. TENNER-RACZ: It's really very difficult to find giant cells in frozen slides. You can't prove on the frozen section whether it contains viral proteins or not, but in one case we did and they were positive. We did see a multinucleated giant cell with heavy positivity with core protein.

DR. ZIEGLER: We can leave this subject now because it's still definitely an open question, the cytopathology of the virus or viral proteins being fusigens. One final point, the CD4 population of lymphocytes is programmed for self-destruction, is it not, because it must be educated by the thymus and self-reactive cells destroyed. They must be set up in such a way that they can be put to death, so to speak, in those subsets that are highly autoreactive. So having already had this time bomb machinery in its genome, it may be additionally susceptible in the body to other cytopathic events. Do you agree with that, Arthur?

DR. GOTTLIEB: I'm not sure I totally agree with that. I think it points up another lack of critical information on the lifetime of CD4 cells in the normal individual and the lifetime of CD4 cells that are in a varying state of viral interaction, whether they be infected, latent, or productive. We need to get that sort of information. I would be surprised if all CD4 cells self-destruct, but quite honestly, I just don't think we know.

DR. ZIEGLER: Would anyone else like to make any comments? One thing that intrigues me is what happens to the dendritic cells? How do they die? Where do they go? Are they killed by CD8 cells that have moved into follicles? It's an intriguing phenomenon and really utterly a black box as far as pathogenesis.

DR. SHIELDS: In the first slide shown by Dr. Racz, you showed a germinal center in which you had it almost cut in polar axis and the cells which stained positively were at the rim of the reactive vs germinal pole. They all seemed to be lined up in one band, and if you really want to study germinal centers with their cell content, if you study them in polar sections, you get quite a different idea than if you sectioned them in random sections. What I wanted to respond to was the fact that probably a certain number of the germinal center cells are infected--that is the B cells--and, they are probably dysfunctional. Now, what is causing them to be dysfunctional? We presume it's virus that integrated into their DNA. If they are dysfunctional, they may produce a lot more antibodies and globulins than normally and not be turned off. This may be why we see this cycling for quite a period of time.

DR. ZIEGLER: Before we turn to Kaposi's sarcoma, our main topic, I want to put one final issue forward and that is the initial infection of HIV. We had some discussion of this at the posters and I wonder--maybe Marlys, you could address this since it forms part of your hypothesis--the transit of the HIV particle from the outside to the inside and here we're talking about really a mucosal disease leaving out transmission by transfusions and dirty needles, and so forth. You brought up at the poster session the notion of the Trojan horse type of transmission where a foreign allogeneic lymphocyte loaded with virus can find its way through the mucosa into the lymphatics. Does anyone want to expand on that or help us with a kind of experiment that would be able to address that directly?

DR. WITTE: You can just address it directly experimentally using foreign lymphocytes. You try to track them, cannulate the regional lymphatics, and try to recover them. It should be possible if you gave a large number of them.

DR. ZIEGLER: Of course, we transfuse foreign lymphocytes all the time in the bloodstream. We don't usually give foreign lymphocytes in the alimentary canal. Ron, do you want to speak to that?

DR. DORFMAN: I'm not sure whether you started this panel discussion with this topic because I was just a little late in getting here, but I was intrigued by Dr. Barre-Sinoussi's discussion of the involvement of macrophages apart from lymphocytes and there's a interesting editorial in the *New England Journal of Medicine*, July of 1987, "Pathogenesis of infection with human immunodeficiency virus", in which the same discussion takes place of the role of the macrophage and suggests many of the clinical syndromes. For example, lymphocytic interstitial pneumonia in children, could be related to infection of the alveolar macrophages, bringing in the role of the macrophage in neural CNS involvement. I think one would have to invoke not only active lymphocyte infection but the macrophage, too, in the pathogenesis of AIDS.

DR. BARRE-SINOUSSI: I think so more and more. I did not discuss too much this morning about those phenomena that were observed but you know that when you try to isolate the

virus from asymptomatic carriers, it's very difficult in fact to isolate the virus from peripheral blood lymphocytes. We're isolating 30-35% of the cases and we try to make a correlation between the clinical status of asymptomatic carrier and viral isolation. When you begin to be able to isolate the virus the prognosis is very bad. In the asymptomatic carrier (i.e. early on) the macrophage is infected and not the lymphocyte from peripheral blood.

DR. ZIEGLER: So what you're saying is that an infected macrophage is not a productive infection and therefore it's very hard to tease the virus out in tissue cultures.

DR. BARRE-SINOUSSI: It does not predict a large number of viral particles, but once a lymphocyte meets a macrophage and if this macrophage is producing the virus, then you would have the spread of infection in the lymphocyte.

DR. RYAN: The macrophages have the T4 receptor, do they not?

DR. BARRE-SINOUSSI: At least some are expressing T4 molecule, some at a very low level.

DR. GOTTLIEB: We need to carefully distinguish two points here. One is the clear ability of the macrophage to be infected, and second, what that has to do with the pathogenesis of the disease. There is an absolute requirement that virus hit a T4 lymphocyte or possibly in other tissues, some other critical cells such as glial cells in the brain, etc., before you get into clinical trouble. It seems to be that the state-of-the-art at the moment basically says that macrophages are reservoirs of infection but what role they play at any stage of the disease process is another unexplored issue. There may be some disagreement on that point.

DR. RYAN: I was going to ask whether the macrophage is carried by human semen as opposed to a lymphocyte. Do we know anything about that?

DR. BARRE-SINOUSSI: Not at all, but I think also in fact macrophages can be infected at the same time.

DR. M. WITTE: From an experimental or actually clinical research point of view, thoracic duct drainage would be most interesting in these patients in regard to the circulating lymphocyte and other immune cell populations. Many of them differ in nature and capability from peripheral blood. You might also be able to redistribute the lymphocytes by selective removal of certain cell types and enhancement of other cell types. It could possibly even be a therapeutic maneuver by preferential enrichment of certain populations or even reduction of viral load.

DR. ZIEGLER: One final comment before we leave this subject and that is, Francoise, would you tell us what your feeling is at the moment about the tropism of the virus? Are we dealing with different strains of viruses that are producing different clinical syndromes?

DR. BARRE-SINOUSSI: It's very difficult to prove, but our feeling right now is that we might have different strains of viruses either in HIV-1 or HIV-2 family, which might have differential tropisms for such cells. The virus that I was speaking of this morning, for example, HIV-1_{PAR}, is one virus that we have found very difficult to propagate in the PBL or in continuous cell line. That is not the first isolate. Several other groups found similar difficulty to propagate some isolates in continuous cell lines. In the future, the tropism of the virus may change and influence what we see.

DR. RACZ: I would like to make two comments. We speak about macrophages but the macrophages have different subpopulations like the lymphocytes. If we stain with CD4, then in the early phase, the macrophages show a very slight mild positivity, but if you have, for instance, a so-called major histiocytosis then the CD4 positivity is stronger like the CD4 positivity of the CD4 lymphocytes. Have you also made this observation?

DR. BARRE-SINOUSSI: Both cells have been shown to be capable of being infected by the virus.

DR. ZIEGLER: We can talk for the better part of the evening on all of these questions. I'm not sure we've even designed the kinds of experiments Marlys was looking for because the issue is so global that just defining our areas of ignorance is probably the best we can do at this point. Let's turn now to Kaposi's sarcoma--the enigma of Vienna as it's been referred to for over 100 years. Perhaps we can start with Michael Dictor who has given us quite a bit of food for thought on the pathogenesis from his vantage point, the microanatomical changes of lymphatic venous anastomoses. Would you summarize again what your point of view is and what you are doing to explore this?

DR. DICTOR: The framework which I presented, of course, is based on angiographic findings and findings of other pathologists before me some of whom have, however, not had access to the angiographic data and have not put together the sequence of evolution of the Kaposi lesion in the manner in which I have. It would be very nice if other pathologists in some of our larger cities who are now seeing many Kaposi cases took a look at the lesion and decided, given all the data that we have access to now, whether or not this is a reasonable summation of what is going on. Of course, we also need further studies defining cell types and their evolution in the Kaposi lesion. What I would like to see happen is pathologists collaborating over borders to gather cases in which clinical immunodeficiency is either well defined or absent and seeing whether the coupling of lymphatics and veins does in fact proceed in the venous direction as I indicated in our comparison between AIDS cases in San Francisco and classical Kaposi's in Sweden. I've also seen a couple of AIDS autopsies in Sweden since arriving there which have been absolutely indistinguishable from classical Kaposi's sarcoma. They have not shown this coupling so I don't think it's a universal phenomenon in our AIDS patients. These radial venal lymphatics which I showed you have been described by other pathologists.

They haven't been specifically recognized, however, as lymphatics. They've been primarily recognized in African endemic Kaposi's sarcoma and in the large series that have been published from Africa covering hundreds of cases; a general immunodeficiency has not been apparent. There have been many cases in which patients had advanced tuberculosis, with parasitic loads and other factors which could conceivably produce a mild clinical immunodeficiency, but certainly nothing like an AIDS epidemic before our time. I think it would be very interesting to go back, as boring as it sounds, and look at this old material in this new light and see whether we can do any sort of correlation in terms of chronic underlying diseases and possibly mild immunodeficiency and the spread of the Kaposi disturbance in differentiation along the lymphaticovenous tree.

DR. ZIEGLER: Can you define more clearly what you mean by disturbance in differentiation? What is the early event here?

DR. DICTOR: One of our problems in pathology (in biology for that matter) is that we always like to define a single cell of origin for any tumor condition. Sometimes we're reasonably successful and other times in many areas of pathology and many tumor conditions, we're less successful. By claiming that lymphaticovenous differentiation is disturbed in the Kaposi lesion, I neatly avoid the question of having to pinpoint a specific cell as the origin for both venous endothelium and lymphatic endothelium. We're still undecided as to how the lymphatic system originates in the body and we still have opposing views, unless it's been ironed out by the International Society of Lymphology, as to whether lymphatic endothelium does in fact in the embryo arise from venous endothelium. We can see when we go back and section embryos in the 20mm or lesser stage, for example, jugular lymph sac structures which very much can be likened to the glomerular structures I showed in the skin. This doesn't necessarily mean that they have the same histogenesis, but unless we're willing to say exactly what the cell of origin is for the lymphatic system, then we don't have to be that precise either in discussing Kaposi's sarcoma.

DR. ZIEGLER: Can you speculate on a general mechanism that can lead us into this? How does a perturbation of the lymphaticovenous anastomoses occur anatomically or physiologically? Why would it occur?

DR. DICTOR: My personal bias is based on a look at the epidemiologic evidence in Kaposi's sarcoma. The primary stimulus for me has been the marked discrepancy in the frequency of Kaposi's in the AIDS epidemic. One goes back and looks at what has happened in both the African and Western World. There is a marked difference in the frequency of endemic Kaposi according to country or perhaps tribal border areas in Africa, and this frequency, although specific data has not been published as far as I know, appears to have dropped in recent years. Another epidemiologic factor is that despite our often quoting the frequency, the sex ratios in Kaposi's sarcoma are also very interesting. They've always been quoted in Western countries as being somewhere between 12 and 15:1. This is based generally on hospital series, and the data is good for about the first half of this century. Since about 1950, our sex ratio in classic Kaposi's has dropped, and, as a matter of fact, right now in Sweden our rate in classical Kaposi is 2.9:1 male to female and that same number almost exactly was found about 2 or 3 years ago in a survey done in America including Puerto Rico by the NIH in which they did a surveillance in several states and found that the Kaposi ratio outside of the context of AIDS was about 3:1. This is probably valid for Western Europe in general although not much has been published. Thus, we're seeing changes in Kaposi's not just in connection with the AIDS epidemic and it's been going on for several decades, both in terms of sex ratio and in terms of incidence. But, it's not a common disease outside of the context of immunodeficiency. Once one gets the bug in their mind about Kaposi's being an independently transmitted disease, you have to relook at what's happening in the AIDS situation. There, we have a low frequency of Kaposi's, under 5%, generally speaking for people who develop AIDS after contact with blood or blood products; that is, in hemophiliacs, probably less than 2% have Kaposi's, in the IV drug users less than 5%, in the post-transfusion patients around 3% or so. If it is indeed caused by a separate agent, this agent is simply being transmitted less efficiently by means of blood contact than by sexual contact. If you draw the parallel further, then the apparent drop in frequency of Kaposi's among homosexuals could be likened perhaps to ships passing in the dark of night in which an AIDS virus and a Kaposi virus could be independently transmitted but showing simply a peak at 46% at the beginning of the epidemic among people who had the most sexual contact. Subsequently as we reach peripheral populations there is relatively less sexual contact, and the differences are more apparent.

DR. ZIEGLER: Are you suggesting that Kaposi's is a sexually transmitted disease that has a high expression in males?

DR. DICTOR: It could be. We have to look at some epidemiologic features of classical African Kaposi's. In our Swedish data over the 25 year period between 1958-1982, we have seen Kaposi cases by far most predominantly in rural people, not in doctors who do cardiac surgery, and not in secretaries who sit at desks. Most of the people come from the countryside including a large number of agricultural farmers, woodworkers, metal workers, builders, and so forth--people who work out in the open. This is a pattern in endemic Kaposi's in Africa. We can only speculate as to whether we're seeing in Kaposi's simply a virus which has changed its transmission pattern in analogy to what we assume to be happening in the transmission patterns of HIV virus. Viruses previously present

at a very low endemic level in many populations have changed their pattern of spread perhaps suddenly, and when combined with immunodeficiency, become unmasked. We have groups of people who are suddenly popping up with Kaposi's sarcoma after receiving steroid therapy, for example, and when the steroids are taken away the Kaposi's often remits. Many of those patients, by the way, who've received steroids have probably also received blood transfusions, often for diseases involved with immunodysregulation such as systemic lupus or dermatoyositis in which pancytopenia is prominently mentioned in the case reports whether or not the patient received blood at any time. But even some of our classical Kaposi patients have received blood at some time in the past. I suspect a "sneaky" virus is involved which we are unable to pinpoint directly but which has remained below the surface and only pops up in situations of immunodeficiency.

DR. ZIEGLER: That's a good introduction to the pathogenesis of Kaposi's because we have to start with epidemiology. If you can't explain the epidemiology, you can't change it.

DR. GOTTLIEB: I have two points to make. One, do you suppose that in classic Kaposi's, cases develop because of immunodeficiency and in older patients as a reflection of aging? When you refer to the studies of immunodeficiencies in such groups where there may not have been a direct correlation, one of the key issues is just what was measured. Perhaps some critical parameters we are now just beginning to be able to measure, for example, suppressor/inducer cells, cytotoxic cells, specific antitumor cytotoxicity, tools needing better monoclonals available now will allow us to evaluate more fully for immune defects at least the cases that are now appearing.

DR. ZIEGLER: You make a good point. We might even start with the definition of immunodeficiency because in early parts of many syndromes associated with infections, the immune system is actually turned on but parts of it aren't working properly. We read this phenotypically as deficiency whereas actually there is a major immunostimulation going on. Chronic malaria is a good example where you have hypersplenism and hypergammaglobulinemia, but youngsters with acute malaria don't respond to tetanus toxoid, reflecting a kind of immunodeficiency. The early studies of immunodeficiency in Kaposi's looked simply at phytohemagglutinin stimulation and skin tests, some very crude measures, and they were basically normal. We took care of hundreds of cases of Kaposi's sarcoma in Kampala in the 60's and 70's and really none of them got Pneumocystis pneumonia or anything else clinically suggesting immunodeficiency, and these patients did quite well with chemotherapy. They very often had good regressions and went home. So, if there was an immune defect, it was exceedingly subtle. Even in the group of homosexual men reported from Los Angeles who were prone to Kaposi's but didn't have HIV infection, there was no evidence of immune dysfunction in Kaposi's sarcoma. We have one or two patients with HIV infection who have totally normal immune function and still have Kaposi's sarcoma. I don't know how to explain these things but the immunodeficiency question is really one of range and it does suggest that there's something else independent of immunodeficiency but perhaps related.

DR. WITTE: I have a number of points. The first is, and I think you put your finger right on it, when you said, how can we really discuss the cell of origin for Kaposi's when we really don't know the origin of even the normal endothelial cell and the relation between the lymphatic and blood vasculature. We just completed a symposium in Tokyo, part of which will be repeated tomorrow here, entitled, *Are Lymphatics Different From Blood Vessels?* and after gathering experts from 14 countries together for an entire day examining anatomical and functional differences in health and disease, regional differences comparing the liver sinusoid, for instance, to the brain capillary, the answer most of the symposiasts gave was that you really can't differentiate them in the general sense, and in diseased states, one can see very leaky blood vessels and highly impermeable lymphatics. For instance, in filariasis you have basement membrane and multiple layers of scar tissue laid down around some of the lymphatics. Unless we can isolate the endothelial cells in pure populations, and this is what we're attempting to do by way of experimental approach, that is to isolate lymphatic endothelium and blood vascular endothelium both normally and in experimental animals and man and then look at the changes that occur in diseased states, you won't be able to approach these questions. I note that Dr. Ryan is the only other person I know of who links such benign disorders as hygromas and hemangiomas with Kaposi's. Most people don't even think that they can even be compared. I've labeled the process "angiotumorigenesis". It seems many more people are interested in "tumor angiogenesis" when perhaps it would be far more fruitful to look at angiotumorigenesis because that might tell us about normal lymphatic and blood vascular endothelium and the proliferative stages. Just one point about lymphatic-venous shunts, and that's to remember that they are normal. The thoracic duct-venous junction in the neck is indeed a lymphtic-venous shunt and they're always available should pressure relationships dictate opening or closure such as by obstruction of lymphatics or veins. But this same obstruction also stimulates endothelial proliferation, which several of us discussed. All you need to do is obstruct the lymphatic system and both lymphangiogenesis and hemangiogenesis are stimulated. Whether this is simply a pressure phenomena unrelated to infection or is dependent on other factors such as oncogenes is unclear. One final point to think about is why Kaposi's occurs almost exclusively in legs in the African form, whereas one sees it on the nose and

upper extremities or trunk or genitalia commonly in patients with AIDS.

DR. DICTOR: I agree that in African's and classic Kaposi's the vast majority of lesions are on the distal extremities especially the legs. I've seen patients just recently with classic Kaposi's in Sweden who have isolated lesions on the elbow, penis, and other parts of the body. It's definitely not two different patterns. We're seeing an overlap and even in most of our AIDS patients, the lesions initially appear on the arms or legs distally. But they tend not to group there for a long period of time and instead you see lesions popping up on the trunk and mucous membranes. We've tried to investigate somewhat this problem in classical cases. For the Swedish autopsies we've collected almost every Kaposi's autopsy performed in the country during a 25-year period and we tried to do a case control using our own autopsy material. We didn't find any correlation to heart weight or to the presence of renal disease--in other words, gross pathology at autopsy that might alter the edema-producing mechanisms in the body. For some unexplained reason, however, we had a slight excess of liver cirrhosis in our Kaposi's patients. But, since the control group is not perfect, I can't make a big point of that.

DR. ZIEGLER: While the distribution of lesions is an important question, I'd like to turn for a moment to the question of proliferation. We're obviously dealing with a neoplasm and vasoformative cell that is growing *in situ* in many places. It seems to me that there's two possibilities. One is that they're driven by some kind of stimulatory factor, or alternatively, some loss of inhibitory factor has occurred in localized areas, or maybe a combination of the two which generates this proliferative lesion. Terence, would you speak to this point?

DR. RYAN: I would like to join Marlys in saying that in Tokyo I was strongly of the opinion that lymphatics and blood vessels should not be too much distinguished. It depends on where they find themselves; in any particular time, they can change from one to the other. I also agree that lymphaticovenous shunts occur normally and in this meeting we've had two examples in posters yesterday. One was in wounds and the other was in grafting in which lymphaticovenous shunts appeared temporarily. I think the fact that Kaposi's sarcoma occurs so commonly in the legs may be related to trauma. In my patients with lymphatic diseases of any kind, they can have a lymphatic disorder in the lymph nodes for a very long time and then a traumatic episode in the arms or legs brings to light that they have a lymphatic problem. Trauma, insect bites, and all sorts of things can precipitate overt evidence of disease in the presence of lymphatic obstruction. This afternoon, Judith Daroczy next door explored my favorite theme that when you have lymphedema the lymphatic endothelium loses its anchorage dependence. She showed as others have done that when you have normal lymphatics, they are well anchored by elastin and collagen fibers but after two weeks of experimental edema, all that anchorage has disappeared. I believe this is due to protease activation and once you've got these cells losing anchorage, they're beginning to behave in a different way. This is a reactive and normal response of endothelum not a malignancy. My final point is that even if I believed that this is normal behavior, I do recognize that we have a disease which is associated with immunodeficiency and sometimes, as in renal transplant patients, the immunodeficiency must come first. Nonetheless, I still believe that the earliest change is probably in the lymph node and then we have a predisposition to get changes in the periphery which become edematous with loss of anchorage dependence of endothelial cells.

DR. ZIEGLER: Do you believe that this protease activation is an important pathogenic mechanism in the development of Kaposi's sarcoma and is this normally inhibited?

DR. RYAN: No, I think this is an area that needs to be looked at because in all other aspects of angiogenesis, plasminogen activator comes out very strong in the story.

DR. ZIEGLER: Ron, did you have something you wanted to say?

DR. DORFMAN: I want just briefly to comment on the fact that there clearly is going to be an emphasis on the use of monoclonal antibodies in the study of malignant tumors of all types and, unfortunately, the tendency to look at tumors in a vacuum. This morning I presented my evidence based on a whole host of observations, clinical, immunohistochemical, enzymatic, radiological, etc., but I did not present any evidence that has been put forward in the literature to the contrary that Kaposi's derives from vascular endothelium which has been done based on, for example, the study of Rutger et al using three unique monoclonal antibodies which apparently identify vascular endothelial cells. One has to look at the disease as a whole and not draw conclusions on the basis of reactivity of cells with monoclonal antibodies recognizing that the behavior of Kaposi's sarcoma is completely different from angiosarcoma with its origin in skin or viscera and its ability to metastasize and kill the patient very rapidly. To me, these are two completely different entities and I would like to be able to separate them.

DR. ZIEGLER: Thank you. That's a good point. Marlys?

DR. WITTE: I think both Terry and I have seen another phenomenon that bears on this. When we were doing the Factor VIII-associated antigen in lymphatics and studied various lymphatic diseases and tumors, there's generally patchiness in staining but one very interesting thing we saw correlates with something Terry saw. We followed a lymphatic out of a cancerous node from breast cancer, and we found a nest of cancer cells and on the side of the vessel where the cancer cells were impinging on the endothelium, Factor VIII positivity was intense. On the other side, it was barely positive at all. Terry noticed that a lymphatic going

into a granuloma became Factor VIII positive. The same structure, just as it was hitting a different environment, changed its staining characteristic. I would predict that monoclonal antibodies are not going to be able to distinguish lymphatics from blood vessels except in a very general sense.

DR. DICTOR: I agree with you, but since most pathologists would like to pigeon-hole Kaposi's as either one or the other, it's going to take a number of other studies before most pathologists come to the insight that you're not going to be able to do it.

DR. ZIEGLER: I would like to ask the pathologists about angiogenesis in lymph nodes. Some of these lymph nodes show considerable vascular reactivity, some have a little bit, and some have none. Would it be a valid experiment or has it been done to look at a correlation between what's going on in the lymph nodes of these patients and the presence or absence of Kaposi's sarcoma? In other words, does a lymph node that has angiolymphohyperplasia give us any clue as to whether it is Kaposi's sarcoma or not? Does this support the notion that angiogenesis factors are important in the generation of Kaposi's sarcoma in the periphery? Can any of the pathologists address this point?

DR. RYAN: It's hard to come back on that, but for instance, take the Stewart-Treves phenomenon. It only occurs in about 1% of lymphedemas and yet they've all got the same background problem so that I think there's always going to be an additional factor we've got to look for.

DR. DORFMAN: There are so many different disorders of lymph nodes which are associated with vascular proliferation. Some of these reactive states such as Castleman's disease, whether localized or so-called systemic angiofollicular lymphoid hyperplasia or angioimmunoblastic lymphadenopathy, is associated with a very prominent arborizing vascularity, but this is a completely different disease and behaves in a much more aggressive fashion. In fact, there's good evidence now based on studies using DNA hybridization, that AILD as it's called, is a T cell lymphoma, that there are rearrangements of the basic T cell receptor in both lymphomas with angiogenesis and in the AILD-like picture. So, it's hard to know how to interpret vascular transformation in lymph nodes because it's such a common phenomenon in peripheral T cell lymphomas. It seems to bear some relationship to the proliferation of neoplastic cells as well as benign reactive lesions. So, I find it hard to know where to approach this phenomenon in relation to Kaposi's sarcoma.

DR. WITTE: I've one question that should have an answer to it but I don't think I'm going to get one and that is, does NIH have a Kaposi's sarcoma endothelial cell line? Is there one in France? Are these bona fide cell lines or not? Can anyone tell me because I've heard too many rumors and I'm a skeptic?

DR. DICTOR: I don't know of any cell line. A group from Sloan-Kettering has recently published in *Cancer Research* a report of transformation in some Kaposi's spindle cells which they've been able to culture. Most researchers who've tried to culture it find it very difficult. There's also a paper from Paris in which there has been culture of spindle cells and some fibroblasts.

DR. WITTE: How about lymphatic endothelium? Someone from Nancy I believe described an endothelial cell line that was lymphatic in character and I just raise the question whether you are skeptical of these reports?

DR. DICTOR: Well, I would expect if one were to culture endothelial cells from these lesions, lymphatic endothelial and blood vascular endothelial traits would be found because their traits form a spectrum.

DR. ZIEGLER: I think the point is that mesenchymal tissue cultures are notoriously difficult to identify and maintain. Before we leave this, would anyone like to address whether Kaposi's sarcoma really is a neoplasm or not? When we classically think of neoplasia in the process of initiation, promotion, and progression, Kaposi's does fit the bill but we are seeing evidence of polyclonality, multicentric tumors arising simultaneously in different parts of the body. In one sense, these tumors could be viewed as a "rash" in that it comes and goes and doesn't really metastasize in the true sense. Ron, do you want to speak to that question?

DR. DORFMAN: You mentioned this morning the study of Lo and Liotta at the NIH. They approached this controversy by searching for transforming genes associated with Kaposi's sarcoma. If I can just quote from their work, "their findings appear to provide a powerful argument for the neoplastic nature of Kaposi's sarcoma." They reported that "DNA obtained from KS tissue successfully transformed phenotypically normal NIH 3T3 cells. When injected into nude mice, the transfected lines produced vascular tumors which they considered histopathologically similar to Kaposi's sarcoma." I think that last statement needs to be carefully looked at, how similar they are, or whether they're just vascular tumors. But that's the only study that I'm aware of that has been most suggestive of its neoplastic nature.

DR. ZIEGLER: That's a good point, and we have to remember that NIH 3T3 cells are already initiated cells. They're not normal cells. Are there other pressing points or concluding statements?

DR. DORFMAN: We have not in any way discussed the lymphomas that occur in association in AIDS which you so well described some years ago. There are some very interesting recent observations. I heard Daniel Noel's presentation only two days ago discussing his experience at New York University with 106 cases of lymphoma in patients with AIDS emphasizing their incidence in extranodal sites--that they're all B cell lymphomas--that there has been some suggestion of involvement of the c-myc gene in association with the development of these EBV-stimulated lymphoid cells. There's an

enormous amount of information developing on this whole subject of lymphoma.

DR. WITTE: Just a speculation. We've talked about the symbiosis between lymphocytes and endothelium. If you look at a cystic hygroma, it's not a bag of water. It often has what's called lymphorrhage in it, that is, in the embryonic development you see not only endothelium but also lymphocytes. So, is it surprising that one sees lymphatic neoplasms next to lymphocyte neoplasms? Isn't that what the lymphatic system is, all those four elements that we talked about?

DR. RACZ: Concerning HIV infection, tumors, and leukemia, if the observation is true and it is true that the macrophages are also targets, then one can ask whether tumors arising from monocytes and macrophages are observed in greater number. One interesting observation we made several months ago is monocytic leukemia in a patient with HIV infection. *In situ* hybridization showed two HIV RNA expression sites on several percent of the monocytic cells. We should look for tumors of monocytic origin and connections like the B lymphocytic tumors.

DR. ZIEGLER: We're opening up a whole new subject here. But HIV does not contain a known oncogene in its genome although it seems to uncover a number of tumors such as Kaposi's and lymphomas, and perhaps other tumors that will appear if patients live long enough. Whether this is latency or defective immunosurveillance and whether the immune system and its lymphatics let down their guard against neoplasms arising in various parts of the body is still an open question. On behalf of the panel, I would like to thank Marlys Witte for inviting us all here, the Congress for their hospitality, and the panel for their cogent and interesting comments.