Report on the „Third International Conference on Lymphatic Tissue and Germinal Centers in Immune Reactions"

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The third "Germinal Center Conference" was organized by the Department of Histology, University of Uppsala *(K. Lindahl-Kiessling, K. E. Fichtelius, J. Linna, R. C. Hard, T. Brenning, O. Bäck)* in collaboration with the Biology Division, Oak Ridge National Laboratory, Oak Ridge *(M. G. Hanna, jr.)*. In a partial break with the traditional pattern established in the two preceeding conferences, more than 50 papers on topics only partly concerned with germinal center function were presented in 10 consecutive scientific sessions.

Although the proceedings of the meeting will be ready for publication early in 1971, a short annotation on some of the presentations may provide advance information.

Ontogeny and phylogeny of immune responses

E. L. Cooper and *B. S. Baculi* studied the development of immune reactivity in larval anuran amphibians. In larvae older than six months of age, i.e. in a stage of full immunological competence, thymectomy had no effect on the capacity to reject skin allografts. Lymphadenectomized tadpoles, in contrast to thymectomized animals, were unable to produce appreciable amounts of humoral antibody. The authors produced evidence for germinal center formation in amphibians, a developmental step so far believed to have occurred later in phylogeny. With the aid of the newly detected alloantigens Θ and TL which are supposed to be carried by thymus-derived lymphocytes exclusively, *M. C. Raff* and *J. J. T. Owen* tried to assess the ontogenic development of thymus-dependent lymphoid cells in mice. Θ-positive cells were first detected in the thymus on the 15th day of gestation. A gradual loss of the TL-marker and a marked decrease in Θ-activity was taken as a sign of differentiation of lymphocytes in the thymus prior to emigration to peripheral lymphoid organs. Based on the suppressive effect of heterologous anti-μ-chain antisera on the production of IgM and IgG in the developing chicken, *M. D. Cooper, P. W. Kincade* and *A. R. Lawton* proposed a hypothetical model of plasma cell differentiation: the "switch-over" from IgM- to IgG-production occurs in the bursa Fabricii or in the "bursa equivalent" in bursa-less species. According to *N. L. Warner,* lymphocytes in the thymus produce immunoglobulin light-chains only while bursal cells are able to produce both light- and μ-chain activity with subsequent differentiation to γ-chain activity. *A. Silverstein,* in the chairman's summary, stressed the important role of anti-

genic stimulation in the ontogeny of a functional lymphoreticular system: with the exception of the thymus, primitive lymphoreticular structures, including those of the gut, become populated with lymphoid cells only after contact with antigens. In view of the evidence currently available there is still room for the far simpler hypothesis that the different functional capacities of "thymic" and "bursal" cells may be attributed to differentiation, under the influence of antigenic stimuli, of elements belonging to one and the same cell line.

Origin and interaction of immunologically competent cells

M. Feldman, S. Segal and *A. Globerson* used an *in vitro* system to study primary and secondary antibody production to a hapten-(DNP)carrier-(protein, synthetic polyamino acids, bacteriophage)complex. Under the conditions used by these authors, the production of antibody to the hapten was determined by the interaction of at least two cell types: cells recognizing carrier-specific determinants and precursors of cells producing antibodies to the hapten. Information on elusive receptors on immunologically competent cells was presented by *H. S. Micklem* and *C. Asfi* who found an increase in the number of self-reactive, presumably thymus-derived, cells (rosette formation with syngeneic erythrocytes) in lymph nodes of mice following antigenic stimulation. *G. Doria, G. Agarossi* and *S. di Pietro,* based on results obtained in an *in vitro* system using thymus and spleen cells of mice, claimed to have evidence for the interaction between thymus- and bone marrow-derived cells in the induction phase of antibody formation, bone marrow cells carrying receptors of immunoglobulin type. *A. J. Davies* summarized our present state of ignorance with regard to the interaction of macrophages and lymphoid cells of the lymphoreticular tissue in the induction of an immune response, while *J. F. A. P. Miller* further elaborated the current concept of cooperation between thymus- and bone marrow-derived cells in the initiation of antibody formation. According to this concept which is to a large extent based on cell transfer studies in thymectomized and irradiated mice, antibody is produced by bone marrow-derived cells while thymus-derived cells play a helper function in providing antigen-specificity.

Lymphoid cell migration

B. J. Bryant labeled thymic lymphoid cells in guinea pigs by local instillation of a mixture of ^{125}I-labeled deoxyuridine and ^{131}I-labeled uracil of high specific activity. Based on measurements of labeled DNA it was concluded that most newly produced thymic lymphoid cells disappear from both the thymus and major peripheral lymph nodes and spleen with a half life of 2.5 days. A substantial migration of thymic lymphocytes to the periphery, however, was found in calves and mice. *A. D. Chanana, E. P. Cronkite, D. D. Joel, B. H. Waksman* and *R. M. Williams* followed the fate of thymic migrants in the calf either by autoradiography after *in situ*-labeling of lymphocytes with tritiated thymidine via an indwelling cannula in the thymic artery or by immunofluorescence using isoantisera capable of detecting a thymus-specific antigen. Both techniques provided evidence for migration of a substantial number of lymphoid cells from the thymus to peripheral lymphoid organs. *D. D. Joel, M. W. Hess* and *H. Cottier* used local labeling of the thymus to demonstrate almost quantitative migration of thymic lymphoid

cells to Peyer's patches in newborn mice. The traffic of mouse fetal liver cells to the thymus was studied by *O. Stutman* and *R. A. Good.* Thymectomized CBA/- mice were used as hosts for a CBA/T6 thymus graft and for CBA/T6T6 fetal liver cells. Based on the presence or absence of marker chromosomes in mitotic cells it was concluded that primitive hemopoietic cells migrate to the thymus, acquire the capacity to migrate to lymph nodes and serve as the progeny of elements responding to stimulation with PHA or allogeneic cells. According to *J. G. Hall* and *M. E. Smith* the majority of immunoblasts leaving regional lymph nodes following antigenic stimulation localize in the lamina propria of the small gut; lymphoblasts generated at the site of antigen injection appear to reach local lymph nodes via afferent lymphatics where they become associated with germinal center formation. In the chairman's summary, *J. Linna* reported on the migrational pattern of thymus, bursa and bone marrow cells after local labeling with tritiated thymidine in newly hatched chicken. In animals up to 6 weeks of age bursal cells were found to migrate to the thymus, spleen, fecal tonsil and lymph nodes. A similar pattern of migration was established for thymic cells. A significant increase of cellular migration from thymus to bone marrow, from bone marrow to the bursa, and from the bursa to the spleen was noted at 48 hours after antigenic stimulation.

Lymphatic tissue and germinal centers in relation to antibody production

Y. B. Kim and *D. W. Watson* presented a summary of their important studies on immune responses in germfree, colostrum-deprived piglets. Their results demonstrated 1. the dependence of the development of peripheral lymphoid tissue on antigenic stimulation, 2. a sequential production of first 19S and only at later stages 7S antibody in true primary responses, and 3. the association of germinal center formation with 7S antibody formation. With the use of some particulate antigens, macrophages may play an important role in retaining immunogenic material: in the hands of *A. Cruchaud* and *E. R. Unanue* the antigenicity of sheep red cells was retained to 75% in a state associated with macrophage membranes. Summarizing our present knowledge of the role of germinal centers in immune responses resulting in the formation of humoral antibody, chairman *H. Cottier* reported on the appearance of antibody-containing germinal center cells following both primary and secondary antigenic stimulation in mice. Since germinal centers are produced *de novo* in the course of humoral immune responses, antigen-trapping or -retention on dendritic cells in the center of these structures is a secondary event occurring always *after* antibody formation by lymphoid germinal center has been initiated. An important role of the germinal center system seems to be the generation of memory cells.

Localization of antigen and immune complexes in lymphatic tissue with special reference to germinal centers

In studies with flagellar proteins from *Salmonella* organisms, *C. R. Parish* and *G. L. Ada* found evidence for the importance of preexisting antibody on dendritic cells within germinal centers to bring about antigen-trapping in lymphoid follicles. Important to note that these authors also found fragments of flagellin to be localized in the walls

of post-capillary venules at a time when antibody formation was already demonstrable. The role of antigen-antibody complexes in germinal center formation in mice was studied by *J. Laissue, R. D. Stoner, M. W. Hess* and *H. Cottier.* In primary antigenic stimulation immune complexes, prepared *in vitro* at equivalence, were more immunogenic and brought about enhanced and accelerated germinal center formation in regional lymph nodes than antigen alone. In this context, the role of membrane receptors on macrophages and lymphocytes for immune complexes may be of considerable importance. *J. M. Phillips-Quagliata, B. B. Levine, F. Quagliata* and *J. W. Uhr* calculated that about 2×10^6 binding sites for immunoglobulins exist on the membrane of a macrophage. The presence of receptors for C'3 complement components on lymphocytes was demonstrated by *C. Bianco, P. Dukor, R. A. Patrick* and *V. Nussenzweig.* These particular binding sites, present on from 15 to 40% of lymphocytes in peripheral lymphatic tissues, may be related to antigen-trapping in lymph nodes, to the capacity of lymphocytes to respond to chemotactic stimuli, or to facilitation of contact with cells carrying antigen-antibody-complement complexes on their surface. The role of germinal centers in antigen localization was reviewed by *M. G. Hanna jr.* The capacity of reticular cells within germinal centers to retain antigen is brought about by antibody absorbed to their membranes; intricate dendritic processes develop as a reaction to locally formed antigen-antibody complexes. There is abundant evidence that germinal center activity, to a large measure, is directly responsible for the organism's capacity to produce antibodies of the IgG-type and to generate immunological memory.

J. J. Trentin, the invited speaker of the second day, presented his original views on the role of the microenvironment in the differentiation of hemopoietic cells: the eventual development and/or differentiation of a (hypothetical) pluripotential precursor cell depends on the particular "hemopoiesis-inductive microenvironment" (HIM). In the spleen of the mouse, for instance, the areas inductive to erythropoiesis outnumber those favorable for granulocytopoiesis 3 : 1, while in the bone marrow HIMs for granulocytopoiesis are twice as numerous as erythroid HIMs. As a practical example, the author presented evidence for the contention that the Steel mouse suffers from megaloblastic anemia not because it lacks precursor cells but because of a faulty erythroid HIM. These views caution against the all too common readiness to extrapolate results obtained in *in vitro* experiments to the actual *in vivo* situation.

Kinetics of immunologically active cells (models)

Based on results obtained either in *in vitro* or *in vivo* culture systems, hypothetical models on the cellular kinetics underlying immune responsiveness were presented by various authors. *J. Cerny, R. F. McAlack* and *H. Friedman* proposed the following hypothesis: cells of a rather wide specificity with regard to antigenic determinants when in contact with an antigen may generate factors instrumental in attracting and triggering the differentiation of cells producing specific antibody. In this process, the step leading to the appearance of the first antibody-producing cell, according to this model, is independent of the dose of antigen, while proliferation of already differentiated cells occurs in a wave-like fashion and is dose-dependent. A different model, presented by *S. Cohen,* relates the induction of tolerance or antibody formation to the number of

membrane receptors on competent cells occupied by antigen: antibody formation occurs only when the cell is activated by the occupation of a critical number of receptor sites; binding of antigen to a number of receptors below the critical level would lead to "low zone tolerance", overloading to "high zone tolerance". *J. Sterzl* expressed similar views based on results obtained with computer models programmed with the S-X-Y-Z schema of proliferation and differentiation of immunologically competent cells. The most interesting aspect of *Sterzl*'s model is the idea that immunologically active cells (Y, PC_2) differentiate to antibody-producing cells (Z) only when in contact with antigen; if no antigen contact occurs, these Y cells become memory cells. Consequently, antigens which are metabolized or excreted at a very slow rate (e.g. pneumococcal polysaccharide) may lead to a gradual loss of "memory".

Regulation of lymphatic tissue function and *modification of immune responses by external agents with special reference to germinal centers*

These two sessions will be considered together since the topics considerably overlapped. *I. R. Cohen* and *M. Feldman,* in an *in vitro* assay of cell-mediated immunity *(Ginsburg* method), demonstrated that sensitized lymphocytes are activated by specific antigen to cause target cell destruction by a cytotoxic process which itself is not antigen-specific. Mechanisms underlying, and the degree of, immunological incompetence of Snell/Bagg dwarf mice were discussed by *R. J. Duquesnoy* who found that prolonged nursing helped these animals to gain normal capacity to perform cell-mediated immune functions. *C. D. Baroni* proposed that these pituitary dwarf mice suffer from a relative lack of 19S antibody-producing cells, while *W. Pierpaoli* insisted on the beneficial effects of growth hormone in rendering these animals immunologically competent. In a summary of the papers dealing with innate control mechanisms of immune responsiveness, *J. W. Uhr* presented a review on feed-back control of antibody synthesis by humoral IgG. Antibody levels in hyperimmunized rabbits started to rise if their own plasma was returned to them following depletion of antibody by immunoabsorbance. Since a stimulating role of retained antigen could not be excluded it was proposed that the dynamically less favored reaction of antigen with specifically sensitized cells was far more effective than the favored reaction of antigen with antibody, resulting in an increased production of antibody-forming cells.

The problems involved in any attempt at modifying immune responsiveness by external agents may be visualized to center around 1. the target cell system, 2. the immunosuppressive agent, and 3. the environment in which target and agent interact. *M. W. Hess* and *R. D. Stoner* reemphasized the discrepancy between the complexity of the very system to be influenced and our ignorance with respect to many mechanisms governing its functional integrity. A critical look at some immunosuppressive agents currently in experimental or even clinical use reveals a similar lack of knowledge regarding pharmacodynamics, action and side effects. Ionizing radiation is still the best controlled measure of external immunosuppression. *C. Rosse, R. Tyler* and *N. B. Everett,* in a detailed study on cellular recovery in lymphoid tissue following whole body irradiation in guinea pigs, established a complex pattern of proliferation and restoration of normal lymphoid cellularity. Recovery of the lymphoid appearance of the thymus preceeded that of peri-

pheral lymphoid organs; repopulation of lymph nodes exposed to antigens (mesenteric and cervical nodes) was more rapid than that of more "oligosynthetic" structures (popliteal and axillary nodes). The influence of heterologous antisera to macrophages and lymphocytes on the overall immune capacity of mice was reviewed by *R. Gallily*. A possible correlation of the immunosuppressive effects of l-asparaginase with lymphocyte production in the thymus was pointed out by *M. E. Weksler* and *B. B. Weksler:* this bacterial enzyme may be effective through depletion of asparagine, thus inhibiting glycoprotein synthesis and thymic lymphocyte proliferation (the thymus contains 30% more asparagine than spleen on a weight basis). At the end of the session the chairman expressed his hope that in the future we should be in a position to monitor the proliferation and differentiation of cells of the lymphoreticular system without life-threatening interference with the function of other vital organ systems.

Tolerance and autosensitization

M. A. B. de Sousa and *J. H. Humphrey* observed a faster development, and seemingly preferential population with lymphoid cells, of lymph nodes in the draining pathway of tolerance-inducing antigen injections in newborn mice; the capacity to produce antibodies to antigens unrelated to the "tolerogen" was increased. Provided that tolerance was really established, this observation may be explained on the basis of 1. an active antibody response to antigens contaminating the "tolerogen", or 2. a block in the differentiation of lymphoid cells proliferating in response to stimulation but unable to synthesize specific antibodies. An indication of validity for the hypothesis that tolerance may be due to an exhaustion of cells able to react to a given antigen was contained in the presentation of *D. Nachtigal:* rabbits, injected with human serum albumin following X-irradiation, could be switched from a state of tolerance to a state of active antibody production and back to tolerance by changing the route of administration and the physical form of the antigen. Since the number of rosette-forming (= presumably antigen-reactive) cells in the spleen of tolerant mice was similar or higher than in normal or immunized animals, *O. Sjöberg* speculated that initiation of tolerance affected mainly cell lines destined to produce high-affinity antibody. According to *W. O. Weigle,* chairman of the session, the tolerant state is characterized not by a lack of precursor cells for antibody production but by a deficiency in antigen-handling. In the nomenclature presently in fashion, low-zone tolerance may be due to faulty function of "thymus-dependent" cells while immunological paralysis (or high-zone tolerance) results in an exhaustion of memory cells as well.

Neoplastic disease and the immune system

While evidence for immune reactions of the host against tumor cells is present in many cases of neoplasia, neoplastic elements in general manage to escape destruction. The reasons for this apparent inefficiency of the lymphoreticular system were summarized by *P. Alexander* who stressed the complexity of the overall immune response in respect to different types of reaction involved which in turn are not at identical levels in every part of the organism. Humoral antibody directed against antigenic determinants of tumor cells may be instrumental in preventing blood- or lymph-borne metastases. Direct

killing of tumor cells is brought about by macrophages and, most efficiently, by sensitized, cytotoxic lymphocytes. Sensitization of lymphocytes, induceable *in vitro (C. F. McKhann)* and demonstrable *in vivo (J. Stjernswärd* and *F. Vanky),* however, is not sufficient for protection: under certain conditions sensitized lymphoblasts, for reasons unknown, appear to be unable to leave the site of their production (regional lymph node). The role of humoral antibody, known to inhibit lymphocyte cytotoxicity *in vitro,* has not been elucidated in this respect.

At the end of the conference, it was decided to hold the next meeting in Yugoslavia in 1972; by acclamation, the organization was put in the hands of *B. J. Jankovic* and his collaborators.

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Lymph and Plasma Proteins: Barriers to their Movement throughout the Extracellular Fluid*

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During the development of the mammalian organism two vascular systems are formed in most tissues. The fluid they contain, blood plasma and lymph, together with that in the intervening connective tissue, comprises the extracellular fluid or the "milieu intérieur" of *Cl. Bernard* (5). Each system is lined by endothelium which is supported in the larger vessels by layers of connective tissue and smooth muscle cells: in the smallest vessels, however, the endothelium usually has little support except a basement membrane, and even this may sometimes be absent. It is, therefore, mainly by exchanges through the thin walls of these small vessels that the cells of the body maintain their normal metabolism. Substances of small molecular size very rapidly exchange by diffusion; the macromolecules such as the proteins and lipoproteins move much more slowly from compartment to compartment throughout the extracellular phase.

Towards the end of the last century, the mechanisms concerned in the formation of lymph were critically debated (40). However, there has evolved over the years a concept which has been fairly generally accepted. It embraces the view that lymph from any tissue contains all the proteins that can be detected in plasma and that lymphatic vessels are, in general, essential for the continual movement of these proteins in one direction throughout the extracellular fluid of the body – from plasma to tissue fluid to lymph and back to the plasma. In the course of a day in man, protein equivalent to about 25 per cent or more of the total extracellular fluid proteins leaves the blood vascular compartment and an equivalent amount is returned to the plasma in the lymph; in some

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