References

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Acknowledgement: The author wishes to thank all the contributors. Without their help this report would not have been possible.

Report on the "Second International Conference on Germinal Centers of Lymphatic Tissue", held in Padova, Italy, June 26-28, 1968

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Lymphology 1, 120–127 (1968)

The second "Germinal Center Conference" was aptly organized by the Institute of Pathological Anatomy of the University of Padova (Drs. L. FIORE-DONATI, L. CHIECO-BIANCHI, N. PENNELLI, G. TRIDENTE, G. M. CAPPUZZO, D. COLLAVO, and Mrs. P. SEGATO) under joined sponsorship by the Consiglio Nazionale delle Ricerche, Rome (Italy), and the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. (USA). Patterned much after the successful first conference it offered an opportunity to about 100 participants from Europe, the United States and Australia to report and discuss their newest scientific findings.

A total of 53 papers were presented in 15 scientific sessions. The duration of each presentation was limited to 10 minutes, thus allowing ample time for discussion following each session. Abstracts submitted by the authors will be published in "Experimental Hematology" (Biology Division, Oak Ridge National Laboratory); the proceedings of the conference, including comments and discussion remarks, will be edited by the organizers and should appear in print at the beginning of next year. The following summaries of some of the papers presented may serve as a quick inventory of the topics covered at the meeting.

Session 1: *Development of Lymphoid Tissue and Germinal Centers in Relation to Phylogenesis.*

Comparative studies of immunological capacity and lymphoid tissue morphology in several fish species as representatives of the lowest vertebrae revealed that specific 19 S immunoglobulins (IgM) may be produced by animals lacking germinal center structures (B. POLLARA, J. FINSTAD and R. A. GOOD, Minneapolis, Minn., USA). By amino acid sequence analysis of the antibody produced by these primitive fishes, homologies to gammaglobulins in mouse and man could be detected. The conclusion

was that the phylogenetically older 19 S immunoglobulin may have evolved long before the capacity to form germinal centers was acquired; germinal center development may have been an essential step towards production of 7 S antibody (IgG). Additional evidence for 19 S antibody production in the absence of germinal center formation in the rabbit was presented by R. A. Good, D. Y. Perey, W. Cain and M. D. Cooper (Minneapolis, Minn., USA). K. E. Fichtelius (Uppsala, Sweden) joined forces with Dr. Good's group in the search for bursa-equivalent lymphoid tissue in bursa-less species, including mammals. Based on results of morphological and kinetic studies, he found that such an "equivalent" may include, in addition to gut-associated lymphoreticular structures, the total of lymphoid cells in close contact with epithelia ("thelio-lymphocytes").

Sessions 2 and 3: *Morphology and Functional Activity of Lymphoid Tissue and Germinal Centers.*

In the hands of F. J. Keuning and A. A. van den Broek (Groningen, Holland), local exposure of a popliteal lymph node in rabbits to 700 rads, followed after 24 hours by a whole-body dose of 450 rads with the lymph node shielded, had no effect on antibody formation against *Salmonella paratyphi B* flagellar antigen. The effect of local irradiation appeared to be overcome by massive repletion of lymph follicles within a time interval of 24 hours. Recovery of homograft rejection mechanisms following higher radiation doses could be effectively delayed by additional thymectomy or local thymus irradiation, while restoration of antibody responsiveness and germinal center activity was less affected by these procedures. These results were interpreted as an indication for thymus-dependence of expressions of cellular immunity *vs.* thymus-independence of humoral antibody formation. P. D. Millikin (Lancaster, Ohio, USA) attempted to take a three-dimensional look at germinal centers by a careful study of serial sections through germinal centers of the white pulp of human spleen. These studies revealed 1) a surprising similarity in the cellular composition of small and large centers, and 2) a distinct polar arrangement of these structures. Because human tonsillar germinal centers exhibit a similar polarity, they were used as a model for studies designed to elucidate the differentiation pathway of immunologically active cells by B. Sordat, H. Gerber, R. Moser and H. Cottier (Bern, Switzerland). Using immunofluorescent and electronmicroscopic techniques it could be shown that the most actively proliferating cells at the base of the germinal center are not participating in immunoglobulin synthesis; IgG was demonstrated in and around cells located at the pole of the center which is oriented towards the epithelial surface.

Session 4: *Cellular Kinetics of Lymphoreticular Tissue and Germinal Centers.*

An extension of earlier studies on the kinetics of tingible body formation in germinal centers of mouse lymph nodes was reported by N. Odartchenko, B. Sordat, M. Pavillard and H. Cottier (Lausanne and Bern, Switzerland). The appearance of labeled tingible bodies and of labeled mitotic figures at from 2 to 60 minutes after a single i. v. injection of tritiated thymidine, cytidine, or leucine was compared. Definite changes in RNA and protein metabolism occur at least during the last cell cycle preceding tingible body formation. The cause underlying this metabolic derangement is not known, but it may be speculated that a direct interaction of antigen with specifically sensitized lymphoid cells could, at least in part, be responsible

for it. R. L. Hunter, R. W. Wissler and F. W. Fitch (Chicago, Ill., USA) studied kinetics and radiation sensitivity of the uptake of ^{125}I-labeled *Salmonella typhi* flagellar antigen and titanium dioxide by dendritic cells located in the lymphoid follicles of the rat spleen. Flagella antigen was found to be broken down and excreted by macrophages in less than 2 days while it appeared to be retained on the surface of dendritic cells for long periods of time. The uptake of flagella by dentritic "macrophages" was dependent not so much on the administered dose of antigen but on the rate of transport of material to these cells. This transport mechanism, and not retention *per se,* was the radiosensitive element in irradiated animals; since attempts to reverse radiation damage by transfer of lymphocytes, monocytes or serum were unsuccessful, stationary structures of the lymphoid follicle may be responsible for effective transport.

Session 5: *Origin and Migration of Lymphoid Cells.*

P. Nieuwenhuis (Groningen, Holland) presented experimental evidence for differential localization in rabbit spleen and lymph nodes of *in vivo* labeled thymic and bone marrow lymphocytes. Reconstitution of radiation-damaged lymphoid tissue by bone marrow-derived small lymphocytes was observed also in thymectomized animals. This finding was interpreted as supporting the hypothesis of the existence of two lines of immunocompetent lymphoid cells: one thymus-dependent, the other directly derived from the bone marrow, i. e. thymus-independent. Conclusions based on the interpretation of highly artificial experimental conditions such as these should be regarded with caution. With the aid of a newly developed method for culturing mouse peripheral blood lymphocytes, A. J. S. Davies, H. Festenstein, E. Leuchars, V. J. Wallis and M. J. Doenhoff (East Grinstead, England) were able to estimate that approximately 80% of the dividing lymphoid cells in the peripheral blood may be of thymic origin. A direct demonstration of emigration of thymic lymphoid cells was given in the following two papers. C. Slonecker, B. Sordat, J. Molleyres and M. W. Hess (Bern, Switzerland) found evidence for the migration of cells from the intact mouse thymus to parathymic lymph nodes. A considerable rise in relative numbers of weakly labeled lymphocytes in subcapsular sinuses of parathymic lymph nodes coincided in time with the highest lymphocytic labeling index at the corticomedullary junction of the thymus; since no indication of an influx of weakly labeled lymphocytes could be observed in the sinuses of more distant lymph nodes, it was concluded that the cells in the subcapsular sinus of parathymic lymph nodes had emigrated from the thymus via lymphatics. T. J. Linna (Uppsala, Sweden) followed the migration of lymphoid cells after local labeling of various lymphoreticular organs with tritiated thymidine by combined autoradiography and direct radiochemical measurements of tritiated DNA. Migration of both thymic cells (in young hamsters) and bone marrow cells (in young guinea pigs) to the white pulp of the spleen could be demonstrated. After local labeling of appendix and Peyer's patches in rabbits, transport of label to tonsils and spleen was evident. A. J. S. Davies (London, England) presented evidence for the participation of thymus-derived lymphoid cells in immune responses. The degree of mitotic activation of thymic lymphoid cells in the paracortical zones of draining lymph nodes could be directly related to the magnitude of immune responses following stimulation with a variety of antigens.

Sessions 6 and 7: *Germinal Centers in Relation to Antigen Localization and Antibody Production.*

Specificity of antigen localization in germinal centers was the subject of a presentation by M. G. HANNA, Jr., M. W. FRANCIS and L. C. PETERS (Oak Ridge, Tenn., USA). While primary retention of ^{125}I-labeled human gammaglobulin in germinal centers of mice may be due to non-specified opsonins, continued localization over a period of weeks and months was dependent on antibody formation. Antigen persisting in germinal centers appeared to play an important role in the development of an anamnestic immune potential. Using the same antigen, P. NETTESHEIM and M. G. HANNA (Oak Ridge, Tenn., USA) studied the radiosensitivity of antigen retention within germinal centers. While exposure to ionizing radiation prior to antigen administration appeared not to interfere with antigen localization, the rate of antigen disappearance from these areas was greatly enhanced in irradiated mice. The interpretation of these results were in general agreement with those offered previously by HUNTER et al. In the hands of D. NACHTIGAL (Rehovoth, Israel), radiation-induced unresponsiveness to stimulation with serum proteins in rabbits is dependent on both the dose of antigen and the frequency of antigen administration during the recovery period following irradiation: with increasing single doses or with more frequent antigenic stimulation, unresponsiveness could be maintained for a longer period of time. F. W. FITCH, R. STEJSKAL and D. A. ROWLEY (Chicago, Ill., USA) followed the appearance of hemolysin and agglutinin containing cells in rat spleen after immunization with sheep erythrocytes; localized agglutination or hemolysis was scored over cryostat sections. In non-immunized rats the occurrence of hemolytic foci was rare but agglutination of erythrocytes was regularly detected over the marginal sinus between lymphoid follicles and the marginal zone. During the early 19 S antibody phase after a single intravenous immunization, areas of localized hemolysis or agglutination were confined to the periarteriolar lymphoid sheaths and the areas surrounding the marginal zone. A correlation of 7 S antibody formation with hemolysin and agglutinin activity over germinal centers could be demonstrated. Working with a similar technique, I. YOUNG, J. ALLEN and H. FRIEDMAN (Philadelphia, Pa., USA) studied the appearance of cells releasing antibody against *E. coli* endotoxin in spleen sections of mice. Bacteriolytic foci, present in low numbers also in 2-week-old non-immunized mice and increasing in number following immunization, corresponded to lymphoid follicles. Since the immune response to endotoxin is considered to be 19 S, the correlation of 19 S antibody forming activity with germinal centers is somewhat in conflict with other results reported at the meeting. The role of antigen-antibody complexes in the initiation and maintenance of immune responsiveness is still widely unknown; experiments, such as the ones reported by G. TERRES and A. H. COONS (Boston, Mass., USA) are very important and may lead eventually to a better understanding of both primary and secondary antibody formation. Mice were primed with bovine serum albumin, complexed at equivalence with isologous antibody, and secondary injections of antigen alone were given at different time intervals after primary stimulation. Whereas an enhanced secondary response could be elicited 4 days after the administration of complexed antigen, little or no anitbody was produced when the second antigen injection was given from 8–10 days after primary stimulation. The

combined histological and immunofluorescent examination of spleen sections revealed a correlation of enhanced secondary responsiveness with greatly increased numbers of antibody-containing plasmacytoid cells shortly after secondary antigenic stimulation. The question of whether or not stimulation with immune complexes might simulate an early anamnestic response could not be resolved.

Session 8: *Role of Central Lymphoid Organs in Germinal Center Formation.*

To obtain further information on the relationship between the bursa of Fabricius and germinal center formation in chicken, B. D. Jankovic, K. Isakovic and D. Vujic (Belgrade, Yugoslavia) grafted 12-day-old chicken embryos *in ovo* with pieces of thymus, bursa or spleen from adult donors. After hatching, some of the birds were bursectomized. All animals were immunized with human erythrocytes at the age of 4 weeks, and a booster injection was given 30 days later. Non-operated birds, grafted with bursa tissue, had an increased number of germinal centers in the spleen and particularly in the thymus. Bursectomized birds, implanted with either bursa or thymus, exhibited an increased number of germinal centers in the spleen and in the cecal tonsil (but not in the thymus) as compared to bursectomized chickens hatched from non-grafted eggs. Grafting of embryos with splenic tissue had no influence on germinal center formation. The rate of hemagglutinin formation could not be correlated with the number of germinal centers formed. M. D. Cooper (Alabama, Ala., USA) studied the effect of prednisone or 6-mercaptopurine treatment on the development of IgG and IgM formation in the chicken. Both drugs appeared to cause a delay in the normal increase of IgG serum levels in the developing chicken; treated animals were able, however, to respond to stimulation with sheep red cells. A possible relationship between the thymus and germinal center formation was demonstrated by J. Laissue, M. W. Hess, R. D. Stoner, H. Riedwyl and H. Cottier (Bern, Switzerland, and Upton, N. Y., USA). Number and size of germinal centers in popliteal and mesenteric lymph nodes of neonatally thymectomized mice were studied following secondary stimulation with tetanus toxoid. Both number and size of germinal centers formed in regional lymph nodes of thymectomized animals were significantly reduced as compared to sham-operated controls. The difference between the two groups was less striking with regard to germinal center formation in more distant lymph nodes: a reduction in the number, but not in the size, of germinal centers was noted in thymectomized mice. These results were interpreted as indication of a direct influence of the thymus on the availablity of socalled "germinal center forming units" with regard to the ability of anamnestic immune responsiveness.

Session 9: *Germinal Centers in Delayed Sensitivity.*

There appears to be little or no connection between germinal center formation and cell-mediated immunity according to the presentations of D. M. V. Parrot (Glasgow, Scotland) and J. L. Turk and J. Oort (London, England, and Leiden, Holland). It was suggested that germinal center cells and plasma cells may belong to a functionally and anatomically distinct system within lymph nodes, associated exclusively with humoral antibody responsiveness; lymphoid cells in the paracortical area of lymph nodes (i. e. belonging to the recirculating and mobilizable cell pool) would be associated with cell-mediated immunity. Experiments on cellular aspects of homo- and xenograft rejection in rats provided further support of this concept (R. Bilski and

C. JERUSALEM, Nijmegen, Holland). A method which may become useful in the clinical assessment of graft rejection was described by H. S. MICKLEM and N. A. STAINES (Edinburgh, Scotland). Nucleated cells in lymph nodes draining the area of a skin allograft in mice were incubated with skin donor-type erythrocytes. An increase in the number of rosette forming lymphocytes ("alloclusters") was found from the 6th day after grafting, and a maximum (15- to 100-times more) of rosette forming cells could be detected after 10 days.

Session 10: *Effects of Immunosuppressant Agents on Lymphoreticular Tissue.*

In 3 out of 4 papers presented during that session, the effects of antilymphocyte serum (ALS) on lymphoreticular organs were described. In mice (L. FIORE-DONATI, G. M. CAPPUZZO, D. COLLAVO, N. PENNELLI and L. CHIECO-BIANCHI, Padova, Italy; G. TRIDENTE and D. W. VAN BEKKUM, Riswijk, Holland) and in dogs (O. COSTACHEL, I. CORNECI, T. ANDRIAN, A. FETEANU, N. VOICULET and G. MALTEZEANU, Bucharest, Rumania) a striking decrease and near disappearance of lymphocytes belonging to the mobilizable pool was noted following ALS treatment. P. DUKOR and F. M. DIETRICH (Basel, Switzerland) found that the *in vivo* blast-transformation of lymphocytes following local injection of phythemagglutinin in mice may be abolished by either neonatal thymectomy or by treatment with one of various immunosuppressive compounds (cyclophosphamide, 6-mercaptopurine, azathioprine, cortisone).

Session 11: *Endocrine Aspects of Germinal Center Control.*

The presentations of this session, both attempting to link pituitary and thymic functions (W. PIERPAOLI and E. SORKIN, Davos, Switzerland; C. BARONI, N. FABRIS and G. BERTOLI, Pavia, Italy) illustrated the enormous need for more detailed information leading towards a more meaningful assessment of hormonal regulation mechanisms in control of morphology and function of lymphoreticular tissue.

Session 12: *Immunological Recovery of Lymphoid Tissue Following X-Irradiation*

A. GLOBERSON (Rehovoth, Israel) studied recovery of immune responsiveness following whole body irradiation in an organ culture system. Spleens of sublethally irradiated mice were cultured in combination with various lymphatic organs, and the recovery of the ability to induce graft-versus-host reactions or to produce antibodies against *Shigella* antigens *in vitro* was studied as a function of time after irradiation. Whereas recovery of graft-versus-host reactivity appeared to depend entirely on the presence of thymic tissue in spleen explant cultures, the ability to form antibodies required the additional presence of macrophages. A possible dual role of bone marrow lymphocytes as stem cells for either erythropoiesis or immunocompetent cells was demonstrated by G. DORIA (Rome, Italy). Lethally irradiated mice were protected with isogenic bone marrow cells and transfused with isogenic erythrocytes to depress erythropoiesis. These transfused animals, when immunized with sheep red cells at appropriate time intervals after irradiation, showed decreased erythropoiesis in combination with enhanced immune responses. Experimental results of M. M. SIMIC and M. Z. PETROVIC (Belgrade, Yugoslavia) supported the concept that circulating lymphocytes repopulate and restore the immune capacity of locally irradiated lymphoreticular organs.

Session 13: *Functional Selection of Lymphoid Cell Populations.*

An interesting hypothesis of germinal center function was presented by B. Pernis, M. Governa, R. Scelsi, E. Maura and M. Ferrarini (Genova, Italy): based on the finding that antigen-antibody complexes located within germinal centers are unable to fix complement, it was postulated that antigen receptors on proliferating lymphoid cells could have a much higher affinity for antigen than free antibody. Germinal centers may thus represent sites where cell clones are selected which produce antibody of progressively higher avidity in the course of continued or repeated antigenic stimulation. Further support for clonal specificity of germinal center activity was given by R. Cepellini (Torino, Italy). A marked variation in antibody titers obtained in different fragments of a single lymph node was observed. Electrophoretic analysis of the antibody produced by each fragment revealed distinct bands which resembled monoclonal bands of myeloma proteins. J. F. A. P. Miller (Victoria, Australia) attempted to identify the participants in the complex cellular interactions which characterize an immune response. From the results of intricate cell transfer studies he concluded that immunocompetent bone marro cells may acquire antigen-sensitivity in the thymus. A minority population of antigen-sensitive cells may be recirculating and is present in the thoracic duct lymph. Although capable of antibody production, these bone marrow-derived thymic lymphocytes appear to contribute very little to the total amount of antibody formed following antigenic stimulation; according to Miller their main function may lay in a synergistic action on antibody forming cells from the bursa equivalent. It appears to become increasingly difficult for the uninitiated to interpret the meaning of complex cell transfer studies.

Session 14: *Germinal Centers in Human Pathology.*

Germinal center formation and activity was found to be increased in the spleen in cases of idiopathic thrombocytic purpura (L. Câmpeanu, B. Wechsler and C. Penea, Bucharest, Rumania) and in the jejunal mucosa in cases of infectious hepatitis (G. Astaldi, R. Airò, M. C. Conrad, R. Penna and F. Cerotto, Milan, Italy, and Washington, D. C., USA). C. C. Congdon (Oak Ridge, Tenn., USA) postulated the existence of a close analogy between the fine architecture of germinal centers and tubercles in granulomatous conditions; scarcity of information precluded the postulate of a similar analogy between non-tubercle types of granulomatous tissue and the diffuse cell proliferation in cell-mediated immune responses.

Session 15: *Germinal Centers in Neoplastic Disease.*

In this final session, C. Jerusalem (Nijmegen, Holland) described germinal center formation and eventual malignant transformation in the thymus of Swiss mice which were immunized with *Plasmodium berghei.* P. Burtin, V. Loisillier, D. Buffe, M. Guillerm and E. Gluckman (Villejuif, France) studied immunoglobulin production in germinal centers of pericancerous lymph nodes in man. The amount of immunoglobulin produced could not be correlated with either presence or absence of metastatic cells in the node. IgA was the most frequently encountered immunoglobulin class; in view of the inability of IgA to fix complement, hence its lack of cytotoxicity, this finding may be of clinical significance.

The meeting closed after a general discussion during which all participants were given an opportunity to restate their respective viewpoints on various inconsistencies which had not been resolved during lunch or dinner recesses.

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Lung Function Studies after the Intralymphatic Injection of Emulsions of Ethiodol in Dogs

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Lymphology 1, 127–131 (1968)

Originally we became interested in developing an emulsion of Ethiodol in an effort to speed up the procedure of lymphography. With the viscous Ethiodol 45 to 60 minutes are required for injection. Certain low viscosity emulsions can be prepared, allowing much more rapid introduction. These emulsions are equal or superior to Ethiodol in definition of lymphatic structure and certain of them are equally long retained in the nodes for long term radiographic studies (3).

The search for an emulsion takes on added importance since Ethiodol globules embolize in the lungs to greater or lesser degree when lymphography is performed in man or animal, the globules reaching the lungs via the thoracic duct or by lymphatico-venous anastomoses. In most instances this is of no consequence but rarely a patient with concurrent lung disease or with lungs damaged by prior disease or radiation develops acute respiratory difficulties. Unassailable evidence of decrease in pulmonary diffusing capacity after lymphography has been obtained and this appears to be mainly due to a decrease in pulmonary capillary volume (5, 6). Pulmonary oil embolism ought not to occur if the particles of the emulsion are below the diameter of a lung capillary. Pulmonary function following lymphography with Ethiodol emulsions was therefore examined using the dog as an experimental animal.

Methods

Pulmonary function studies were performed on twelve dogs in the laboratory before lymphography, within the first two hours postlymphography, and at 24, 48 and 72 hours and 7 days after lymphography.

The dogs were of both sexes and weighed 14 to 20 kg. All pulmonary function tests were performed under Nembutal anesthesia. Expired gas was collected in a Douglas bag for 3 minutes and arterial blood samples were obtained simultaneously. Diffusing capacity was determined in duplicate using the method of Ogilvie, et al. (8). One liter