Meetings

Cellular Differentiation in the Immune System

The theme of the Eighth Midwinter Conference of Immunologists, held in Pasadena, California (26–28 January 1969), was Regulation of Cellular Differentiation in the Immune System.

Ray Owen (California Institute of Technology) chaired the first session on "Principles of Cellular Differentiation." This session provided a very fruitful interaction between two speakers disciplined in embryology and an audience who were specialists in immunology. In presenting "General Aspects of Cellular Differentiation" Clifford Grobstein (University of California, San Diego) focused on three topics. The first was concerned with replicability of the differentiated state, and was presented within the framework of the current concept of three stages of cellular development: I-Primitive, II-Stem (replicating), and III-Differentiated. Synthetic activity in the form of DNA synthesis is characteristic of I and II, specialized mRNA of II, and specialized protein of III. In specific examples of cell and product, interconvertibility among the three cell types was shown. Under a second topic, the relation of replication to transcriptive pattern, various parameters were described by Grobstein as influencing the occurrence and amount of cell product. One parameter, hormonal environment during replication, determines subsequent synthetic activity by cells. The third topic was concerned with cell-to-cell interaction, exemplified first with epitheliomesenchymal interaction in embryological rudiments and second with immunoglobulin synthesis. The latter was the first mention of cellular interaction in an immunological sense but this topic became so frequent in subsequent presentations that it may be considered a main subtopic of the conference. The intertwining of embryology and immunology was indicated in Grobstein's closing remark that both disciplines seek answers to similar questions of differentiation, for example, the kind of regulation that occurs in cells (distant, hormonal; central, neuron; and so on) and the nature of the cue, as well as the steps, in the instructive pathway.

The second speaker, Robert Auerbach (University of Wisconsin, Madison), concentrated on "Cellular Differentiation in the Immune System." After presenting examples of development in vitro (for example, lens), where differentiation is dependent upon the interaction of three cell types, Auerbach proceeded to similar immunological models of cell interaction—requirement of thymus and bone marrow for restoration of immunocompetence after lethal irradiation of spleen, a tissue which normally contains at least three cell types; aggregation phenomena in suspension cultures; importance of cell density rather than cell numbers with the implication of product as limiting; and increase in plaque number in follicles as dependent upon surrounding cells. A parallel was shown in development and antibody formation using the stem cell concept, that is: cells limited in number; restrictive differentiation in which the activation mechanism, although unknown, implicated surface phenomena and hormonal control; and differentiation as dependent on tissue site, thus indicating cell multipotentiality rather than unipotentiality.

In introducing the first of the two sessions on "Cells of the Immune System," the chairman, Byron H. Waksman (Yale University) drew attention to the vague and cumbersome terminology of cellular immunology, suggesting that a by-product of the meeting may be better definitions. The first speaker, Ruth Gallily (California Institute of Technology) spoke on "Macrophages," presenting a summarized review of the more relevant information, morphological and biochemical, that characterizes these cells. The immunologic implications of macrophages were discussed from findings by various investigators of biologically active RNA material isolated from macrophages exposed to antigen. Lastly, a detailed description was presented of the speaker's own studies that showed the involvement of macrophages in the immune response when such cells were isolated from non-irradiated animals injected with *Shigella* antigen and used to restore immunocompetence to sublethally irradiated animals.

The subject "Cell Interaction" was discussed by David Talmage (University of Colorado, Denver). The immune response of irradiated mice to sheep red blood cells was shown to be effectively restored with spleen cells in combination with bone marrow cells. These findings were interpreted as implicating more than two cell types. The spleen was described as having two non-adherent cells as well as an adherent cell population. The bone marrow cell was described as antibody-synthesizing but not antigen-sensitive. A three-cell model of the reaction was illustrated with an adherent cell to which antigen attached. In contact with the latter cell were two others, a memory cell with messenger RNA and a unique DNA. While possibly forming some antibody, it was this cell that interacted with another, designated as a lymphocyte that had the ribosomal machinery for production of the major amount of antibody.

The second session, concerned with "Cells of the Immune System," was chaired by Karl Hellström (University of Washington, Seattle). Fritz Bach (University of Wisconsin, Madison) introduced the topic of "Lymphocytes and Lymphocyte Transformation" by first mentioning his concern about terms peculiar to his topic. Examples of these terms were "transformation," a term with rather precise meaning in modern genetics but used in a different sense with respect to lymphocytes, and "blastogenesis," a term used to describe the poorly understood process by which small lymphocytes change to large leukocytes in vitro. Lymphocyte stimulants were discussed—for example, general agents. The best known are phytohemagglutinin, antisera against γ-globulin, specific antigens, and allogeneic cells studied in mixed leukocyte cultures (MLC). The frequency of nonstimulation in unidirectional MLC was for siblings 29.2 percent, and for unrelated individuals 0.0 percent. Siblings screened as identical in MLC were similarly identical in their reactions to HL-A leukocyte typing reagents. A

single allelic disparity caused observable differences in the sibling data; quantitative disparity increased when two allelic differences were compared with one. Kinetic measurements indicated a minimal "responding unit" of 1 per 300 cells, a "dividing unit" of 1 per 200 cells, and a cell generation time of 18 hours. Reasons for high frequency of "responding units" in cellular immune reactions and the mechanism for recognition were discussed.

The second speaker, Eli Sercarz (University of California, Los Angeles) elaborated on in vitro experiments that were directed toward substantiating his previously formulated X-Y-Z scheme of maturation of immunocompetent cells. Briefly, this scheme involves an X cell or antigen-sensitive lymphocytic cell which, upon antigen stimulation, is converted to a Y or "memory" cell; the latter, triggered by antigen, divides and matures irreversibly to a Z or terminal cell that is a mature, antibody-producing plasma cell. Using a constant in vivo dose of 10 mg of bovine serum albumin per rabbit and exposing spleen tissue in vitro to varying levels of antigen, different levels of unresponsiveness were obtained. While the data established the existence of a reversible state of in vitro paralysis, the cell stage at which paralysis occurs remains unknown. The time for establishment of memory in the primary response was less than 1 day. During this time, no cell division was required, but during the first day of the secondary response progenitor cells divided. It was concluded that it may be possible to study directly the antigen-binding activity of memory cells by sensitive techniques of micromanipulation, specific enzyme-binding antibody, and fluorimetry.

Robert Good (University of Minnesota, Minneapolis) chaired the session on "Cell Population Qualities and Kinetics in the Immune Response." Edward Boyse (Sloan-Kettering Institute for Cancer Research) spoke on "Antigenic Differentiation of Lymphoid Cells" and primarily about his own investigations. This work deals with normally occurring surface antigens, involving genetic differences among cells of the same type (for example, thymocytes) from different individuals, or phenotypic differences among the cells of a single individual. Whereas the genetic differences are of practical significance in homotransplantation, the phenotypic differences are of special interest because they are presumably relevant to the organization of interdependent cell populations within an individual. Six antigen systems have been defined for mouse thymocytes, using cytotoxic antisera in an in vitro test. The cell surfaces have been mapped on the principle that when antibody is absorbed by one of two cell antigens of different specificity, both situated in close proximity on the cell surface, the subsequent absorption of antibody by the other antigen is impeded. All the antigens studied occur on both thymocytes and lymphocytes with the exception of one set, TL, that is present only on thymocytes. Normally the antigens are stronger on thymocytes than on lymphocytes except H-2 which has four times as much on lymphocytes as on thymocytes. The genes for H-2 and TL are linked; the others are independent in inheritance. The TL genes (*Tla*) are unexpressed in TL− mice, but may be activated during leukemogenesis. Leukemia cells taken from immunized animals do not express TL antigen, but the cells regain the antigen upon passage in nonimmunized syngeneic hosts, a phenomenon termed

antigenic modulation. The features of antigenic modulation were amplified. It is assumed, but not established, that the cluster pattern of the antigens is a basic repetitive unit on the cell membrane.

Takashi Makinodan (Oak Ridge National Laboratories) spoke about "Kinetics of Cell Populations" and described various models of cell multiplication that might account for the exponential increase in numbers of antibody-forming cells. The experimental system of this investigator involves spleen tissue from mice stimulated with sheep red blood cells. Although the rate of cell increase is actually dependent upon antigen dose and factors such as cell migration, death, and possibly dedifferentiation, the models were simple and did not involve the latter parameters. The models differed with respect to number of recruitments (single or multiple); manner of recruitment (random or non-random); and proliferative capacity of the recruited cells (no division; synchronous or asynchronous division). The data from Perkins and other collaborators at Oak Ridge were most compatible with the model characterized as non-random, multiple recruitment, proliferating synchronously. Recruitment of cells capable of dividing would characterize a highly efficient differentiation process in which only a limited number of programmed cells need be stored at any one time.

The concluding session on "Regulation of the Immune Response" was chaired by Edwin Lennox (Salk Institute, San Diego). Coinvestigators Donald Rowley and Frank Fitch (University of Chicago) spoke on "Feedback Regulation." Model systems where regulation has been achieved were presented and factors involved were discussed from the findings. One model makes use of renal allografts in rats, and was studied for the effect of administering donor lymphoid cells from spleen or kidney (antigen) and/or antibody intravenously at various times with respect to time of the surgery. When both antigen and antibody were administered one day prior to surgery, the grafted kidneys function optimally, over very prolonged periods. Despite kidney survival, the treatment of the host had no effect on survival of skin grafts. Some circulating antibody against donor antigen was detected, even while the kidney grafts were fully functional. Since circulating antibody had not been totally suppressed, the

inhibition of response had been primarily in the delayed hypersensitivity system. When the established kidney was removed, without further treatment of the host, a successful second renal allograft could be achieved. Thus, it appeared that treatment with antigen and antibody had modified host rather than effecting a change in graft. From a second in vivo model involving Sprague-Dawley rats sensitized with sheep red blood cells and Freund's adjuvant specific antibody reduced the delayed hypersensitivity response when antigen was given 9 days later. It was postulated that delayed hypersensitivity involves two interactions between cells of limited number and that one of these (non-macrophage) had been suppressed by antibody. It had been shown previously by an in vitro model (plaque assay) that Sprague-Dawley rats, treated with specific antibody prior to sensitization with 10^8 sheep red blood cells, had a greatly reduced number of plaques in the spleen. The number represented a negligible response compared to well characterized responses to sheep red blood cells over a wide dosage range. It was suggested that, because of the importance of giving antibody before antigen, antibody acts by combining with antigen (at the level of specific antigenic determinants) and its effect is independent of adjuvant effect. Cell interaction appears to be important in both in vivo and in vitro reactions, as evidenced by the fact that the "rocking" of dispersed cells in cultures produced an increased number of plaques. The mechanism for feedback regulation by antibody was suggested as prevention of cell interaction through combination with antigen.

Eugene Lance (Cornell University) gave the concluding speech on "Immunosuppression." Nonspecific immunosuppressive agents (those whose action is not unique to the immune system or to particular antibody responses) include x-irradiation, radiomimetric drugs, steroids, and antimetabolites (all acting on processes of cell division, cell viability, and components of the DNA-RNA-protein sequence). Some uncertain miscellaneous phenomena such as hypnotism, reticuloendothelial blockade and treatment of grafts with RNA were also noted. Specific suppression by antigen and by antilymphocyte serum (ALS) were taken up in some detail. ALS, serum obtained by injecting lymphocytes of one animal into another species (or the antibody fraction eluted after absorption of such

serum to lymphocytes), alters immune reactions profoundly. Some points in describing the scope of the effect of ALS on humoral response were: importance of giving ALS before antigen (optimal effect when given intravenously 3 days before antigen), effectiveness on primary response which is suppressed but not abolished, and noneffectiveness against the secondary response. In order to prolong skin graft survival indefinitely, ALS must be given continuously. The serum caused no change in the thymus but produced selective damage in the paracortical area of thymus-dependent lymphoid tissue (spleen and bone marrow). The fate of labeled ALS-eluted antibody was studied. Because of rapid blood clearance it was assumed to exert a rapid effect. Radioautography of various tissues indicated only a small percentage uptake into lymphoid tissues. Because of large uptake in liver, a model was presented that indicated interaction of recirculating lymphocytes with circulating ALS and clearance of these cells by the liver. Lance expressed concern about the tendency of immunosuppressive drugs and ALS to increase the background of neoplastic cells that might give rise to lymphomas. However, he was optimistic about deriving experimental models using skin grafts and limited amounts of ALS plus other immunosuppressive agents that would lead to avoiding untoward clinical reactions to grafts.

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National Meetings

August

3–6. National **Heat Transfer** Conf., 11th, Minneapolis, Minn. (D. C. Kelly, American Inst. of Chemical Engineers, 345 E. 47 St., New York 10017)

3–7. Society for **Cryobiology**, 6th annual, Buffalo, N.Y. (R. E. Greco, 3175 Staley Rd., Grand Island, N.Y. 14072)

4–5. **Aerospace Structures Design** Conf., Seattle, Wash. (J. R. Fuller, Boeing Co., P.O. Box 707, Orgn. 6-8650, M/S 77-89, Renton, Wash. 98055)

4–5. American Soc. of **Safety Engineers**, College Park, Md. (W. C. Christensen, ASSE, 850 Busse Highway, Park Ridge, Ill. 60068)

4–6. **Deterioration and Preservation of Library Materials**, 34th annual conf., Chicago, Ill. (H. W. Winger, Graduate Library School, Univ. of Chicago, 1116 E. 59 St., Chicago 60637)

4–8. **Molecular Biology and Pathology**, 2nd conf., Saratoga Springs, N.Y. (K. T. Lee, Dept. of Pathology, Albany Medical College, Albany, N.Y. 12208)

5–8. World Conf. on **Records,** Salt Lake City, Utah. (S. E. Beesley, 1030 S. Orchard Dr., Bountiful, Utah 84010)

6–8. Applications of **X-Ray Analysis** Conf., Denver, Colo. (B. L. Henke, Div. of Metallurgy, Denver Research Inst., Denver 80210)

10–13. **Soil Conservation** Soc. of America, Fort Collins, Colo. (H. W. Pritichard, 7515 NE Ankeny Rd., Ankeny, Iowa 50021)

11–13. Symposium on **Crystal Growth**, Washington, D.C. (H. S. Peiser, Room B316, Bldg. 223, National Bureau of Standards, Washington, D.C. 20234)

11–14. Society of **Photo-Optical Instrumentation Engineers**, 14th annual technical symp., San Francisco, Calif. (H. L. Kasnitz, SPIE Symposium, P.O. Box 288, Redondo Beach, Calif. 90277)

12. American **Astronomical** Soc., Albany, N.Y. (G. C. McVittie, Univ. of Illinois Observatory, Urbana 61801)

13–24. Frontier Topics in **Crystallography**, Stony Brook, L.I., N.Y. (E. H. Kone, American Inst. of Physics, 335 E. 45 St., New York 10017)

17–22. **Animal Behavior** Soc., Burlington, Vt. (B. Dane, Tufts Univ., Medford, Mass.)

17–22. American Inst. of **Biological Science**, Burlington, Vt. (J. R. Olive, 3900 Wisconsin Ave., NW, Washington, D.C. 20016)

17–22. American Soc. of **Zoologists**, Burlington, Vt. (J. R. Shaver, Dept. of Zoology, Michigan State Univ., East Lansing 48823)

18–20. **Genetics** Soc. of America, Madison, Wis. (B. Wallace, Dept. of Genetics, Cornell Univ., Ithaca, N.Y. 14850)

18–21. American **Hospital** Assoc., Chicago, Ill. (E. L. Crosby, 840 N. Lake Shore Dr., Chicago 60611)

18–22. New England Assoc. of **Chemistry Teachers**, 31st summer conf., Plymouth, N.H. (M. P. Olmsted, Publicity Chairman, NEACT, 9 Brookmont Dr., Wilbraham, Mass. 01095)

18–22. American Soc. of **Pharmacognosy**, 10th annual, Corvallis, Ore., with **Marine Biomed'cinals** Symp. (P. Catalfomo, School of Pharmacy, Oregon State Univ., Corvallis 97331)

18–22. American **Phytopathological** Soc., Spokane, Wash. (J. P. Fulton, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville 72701)

18–22. National Goals in **Water Pollution Control**, Santa Barbara, Calif. (F. A. Butrico, Coordinator of Environmental Sciences Programs, Battelle Memorial Inst., Columbus Laboratories, Washington, D.C.)

19. **Biometric** Soc., western North American regional, Pullman, Wash. (J. S. Williams, Statistical Lab., Colorado State Univ., Fort Collins)

19–21. **Birch Symp.,** Durham, N.H.

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