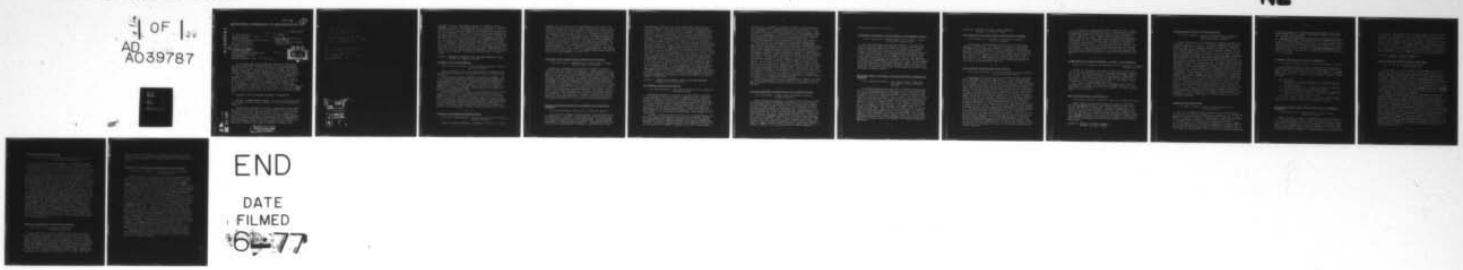
AD-A039 787 CALIFORNIA STATE DEPT OF PUBLIC HEALTH BERKELEY F/G 6/5
MIDWINTER CONFERENCE OF IMMUNOLOGISTS (14TH). HELD ON JANUARY 2--ETC(U)
MAY 75 N E CREMER N00014-75-C-0045
UNCLASSIFIED NL



END

DATE FILMED 6-77

MIDWINTER CONFERENCE OF IMMUNOLOGISTS

Held on January 25-28, 1975 at Asilomar Conference Grounds, Pacific Grove, California.

May 22, 1975

Final report, ONR Contract No. N00014-75-C-0045
Fourteenth Midwinter Conference of Immunologists

Dr. Robert Jennings
Director, Biochemistry Program
DODAAD Code N00014
Biological and Medical Sciences Division
Office of Naval Research
Department of the Navy
Arlington, Virginia 22217

Dr. Natalie E. Cremer
Secretary-treasurer of the
Midwinter Conference of Immunologists
State Department of Health
2151 Berkeley Way
Berkeley, California 94704

The fourteenth meeting of the Midwinter Conference of Immunologists was held at the Asilomar Conference Grounds, Pacific Grove, California, January 25th-28th, 1975. A total of 375 persons attended the Conference, which group included 259 paying registrants, 95 nonpaying students and 21 speakers. There were 5 half-day sessions with four speakers per session. The theme of the Conference was "Soluble Mediators and the Immune Response".

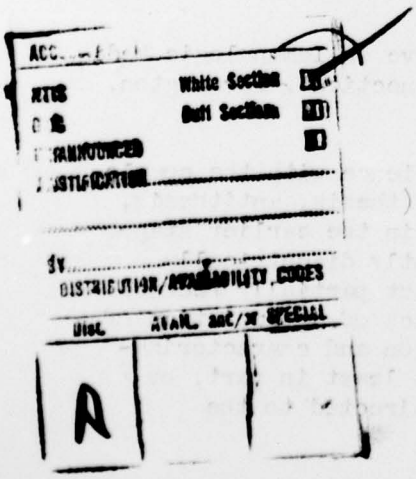
Dr. Dan H. Campbell, one of the three originators of the Midwinter Conference died September, 1974. A special lecture was given in his memory by Dr. Irvine H. Lepow. This lecture was the first in a series to be given annually and to be known as the Dan H. Campbell Memorial Lecture. Dr. Frank Dixon, a cofounder of the Conference with Dr. Campbell and Dr. Hugh Fudenberg, introduced the first lecture with a short commentary on Dr. Campbell as a human being and as a scientist. He spoke of Dr. Campbell's unique ability of relating findings in immunochemistry to biologic phenomena and of his generous giving of his time and council to his peers and students alike.

ABSTRACTS OF THE 14TH MIDWINTER CONFERENCE OF IMMUNOLOGISTS

THE DAN H. CAMPBELL MEMORIAL LECTURE. Perspective on Immunologic Mediator Systems by Dr. Irvin H. Lepow, University of Connecticut, Farmington, Connecticut.

Three examples, selected from the author's experience with the complement system, are illustrative of the Hegelian nature (thesis, antithesis, synthesis) of scientific investigation, particularly in the earlier stages of maturation of a field. Each was marked by apparently diametrically apposed concepts which emerged, in fact, to be at least partially reconcilable. Each suffered from problems of methodology, lack of appreciation of technical details, and need for definitive purification and characterization of the relevant proteins. Each was resolved, at least in part, by independent observations which were not immediately directed to the

Mrs Dorothy Taber
furnished the NR No.
when I called her to see
who the contract was awarded
to
Mr Robert Jennings said
to catalog report to Source
of Contract out Jan 77



controversy at issue. The examples selected and reviewed to the extent necessary to establish these perspectives are: 1) the enzymatic and macromolecular nature of C1; 2) appreciation of cleavage products of both C3 and C5 as phlogistic peptides compatible with designation as anaphylatoxins; and 3) establishment of the properdin system as an alternative pathway of complement activation. Although it is not implied that all scientific controversies are resolved by Hegelian synthesis, the frequency is impressive with which this occurs and is contributed to by the serendipitous interplay of observations flowing from vigorous basic research. In the context of this Conference on soluble immunological mediators, it is also noteworthy, as reviewed in the above examples, that purification and characterization are critically important steps that do not necessarily lead to valid conclusions in the absence of other conceptual input. These perspectives suggest that we seek a greater degree of independence and charity of thought as we continue to attempt to gain more definitive insights into mediator systems.

SESSION I: INTERACTIONS BETWEEN CELLULAR AND HUMORAL MEDIATION SYSTEMS
Chairman - Dr. Charles G. Cochrane

The Hageman Factor Activated Pathways

by Dr. Charles G. Cochrane - Scripps Clinical and Research Foundation, La Jolla, California

Current knowledge of the physical characterization of Hageman factor of human plasma was presented, and the means by which it can be activated described.

Solid-phase and fluid-phase (enzymatic) activation is apparent. The contribution of each remains in question in conditions where Hageman factor is activated in whole plasma. The changes in physical properties of the Hageman factor molecule associated with solid-phase or liquid-phase serve to distinguish these two mechanisms: In solid-phase activation, Hageman factor binds to a negatively charged surface is not cleaved when activated. A conformational change in the structure of the molecule may well occur exposing an enzyme site.

Fluid-phase activation is brought about in greatest part by activated substrates of Hageman factor, a mechanism termed reciprocal activation. Hageman factor (human) is cleaved into 52,000, 40,000 and 28,000 MW fragments in the process. The first two fragments bear the structural groupings responsible for binding of the molecule to negatively charged surfaces. The 28,000 MW fragment is released from the negatively charged surface when the whole molecule is cleaved by fluid-phase (enzymatic) activators. It contains the enzymatic site of Hageman factor responsible for generating activity of the kinin-forming, intrinsic clotting and fibrinolytic systems.

- - -

Complement as An Extracellular Membrane Effector

by Dr. Hans J. Müller-Eberhard - Scripps Clinic and Research Foundation, La Jolla, California

Attack of biological membranes by complement is initiated by enzymatic

cleavage of C5. Activation of C5 is accompanied by dissociation of the activation peptide, C5a, from the α-chain of the molecule. This is accomplished by the multiple subunit enzyme C5 convertase. The enzyme consists of a fusion product of at least three distinct precursors, C2, C3 and C4. The probable subunit structure is C4b, 2a, 3b. Cleavage of C5 constitutes the biochemical signal for self-assembly of the C5b-9 membrane attack complex. Assembly of this multimolecular complex is governed by adsorptive processes. In its nascent state C5b-9 can attach to the outer membrane of a cell and subsequently kill the cell. The complex was isolated and dissociated into its subunits by SDS polyacrylamide gel electrophoresis. Its predicted composition was confirmed, except for the presence of an as yet unidentified protein. C5b-9 is endowed with neoantigens which are unique for the complex and are not shared by its precursor proteins. The probable mode of action of the complex in causing membrane damage appears closely related to the unique quaternary structure of C8.

- - -

Biologically Active Peptides Produced by Neutrophil-bound Enzymes

by Dr. Bruce Wintroub - Harvard Medical School at Robert B. Brigham Hospital, Boston, Massachusetts

Mediators of inflammation may originate from humoral or cellular sources and from cellular-humoral interactions. Enzymes supplied by neutrophil lysosomes may interact with kininogen, a plasma α-globulin, to generate kinins. In an effort to characterize neutrophil dependent kinin generation, a unique effector pathway, distinguishable from the plasma or cellular kinin system, was detected. This pathway, designated the neutral peptide generating system, provides a mechanism by which the neutrophil may participate in inflammation independent of degranulation and phagocytosis. A diisopropyl fluorophosphate inhibitable protease located on the surface of the neutrophil plasma membrane acts on a heat stable, 90-95,000 M.W. plasma α-globulin to generate a trypsin sensitive neutral peptide which contracts smooth muscle and increases vascular permeability. This pathway is controlled by two normal plasma proteins. α-1-antitrypsin inhibits the neutrophil protease and a Ca++ dependent, 300-350,000 M.W. inactivator destroys neutral peptide. Since the neutrophil protease is detected as an active molecule in unperturbed neutrophils, neutrophil accumulation may outstrip the available α-1-antitrypsin with resultant neutral peptide generation.

- - -

Controls of Secretion from Neutrophils: Complement, Cyclic Nucleotides and Calcium

by Dr. Gerald Weissmann - New York School of Medicine, New York

Human neutrophils release lysosomal hydrolases during phagocytosis or, when phagocytosis is inhibited by cytochalasin B, upon contact with phagocytosable substances (zymosan or immune precipitates). Microtubules were more prominent in phagocytosing than in resting cells, and were observed near primary lysosomes and forming phagosomes. "Regurgitation during feeding" resulted from degranulation of primary lysosomes into newly formed

phagosomes which were still open to the extracellular space as well as from the ingestion of additional material directly into already loaded secondary lysosomes. Both events led to the release of lysosomal contents from intact cells. When granule volume, calculated as the percentage of cytoplasmic volume, was determined by point counting from electron micrographs the fractional volume of granules in resting cells was 22.6% of the cytoplasm. After phagocytosing zymosan for 15 minutes the granule volume was reduced to 9.2%. Pretreatment with PGE_1 (2×10^{-4}M) or colchicine (10^{-5}M) reduced degranulation so that the fractional volume of granules in these cells after 15 minutes of phagocytosis was 13.4% and 11.6% respectively. Degranulation was also inhibited by PGE_1 or colchicine when the cells ingested immune precipitates. Degranulation of lysosomes can be studied more conveniently by both morphometric and biochemical methods when cytochalasin B (5 µg/ml) transforms the leukocyte into a secretory cell in which lysosomes fuse directly with the plasma membrane as if to a phagocytic vacuole. Stimulation of cytochalasin B-treated neutrophils with zymosan led to a reduction of granule volume within the cell, and histochemically identifiable myeloperoxidase appeared at the cell/zymosan interface, indicating that lysosomes fused with the plasma membrane instead of with phagocytic vacuoles. As they did in ordinary phagocytic cells, colchicine, vinblastine, PGE_1 plus theophylline, as well as dibutyryl cAMP plus theophylline inhibited fusion of PMN granules with the plasma membrane: e.g. the mean granule volume in colchicine-treated, zymosan-stimulated cells was 13% and 12.8% in cAMP treated cells as compared to untreated, zymosan-stimulated cells which contained a mean of 7.6%. These experiments provide morphologic confirmation of previous suggestions that fusion of granules with phagocytic vacuoles or the plasma membrane may be modified by cyclic nucleotides and the state of assembly of microtubules.

SESSION II: SPECIFICITY AND NATURE OF CELLULAR ACTIVATING MECHANISMS
Chairman - Dr. K. Frank Austen

Cell Receptors: Specificity and Function

by Dr. Pedro Cuatrecasas - Johns Hopkins University School of Medicine, Baltimore, Maryland

Considerable progress has been made in the identification and study of cell membrane receptors for a variety of peptide as well as nonpeptide hormones and drugs. In these studies the general approach has been to measure the interaction (binding) of a radioactively labelled hormone with the intact target cell or with isolated membrane preparations. The binding is surmised to reflect specific receptor interactions if it satisfies certain criteria such as specificity, saturability, tissue and target cell sensitivity, high affinity and reversibility. Although these properties are necessary for the identification of receptors, they alone are not always sufficient to characterize the binding process unless studied in sufficient detail.

A number of problems and pitfalls in the study of hormone receptors are described. Many of these relate to the problem of "nonspecific" binding. It is acknowledged that apparently specific binding of the type expected for receptors can also be seen with materials not containing receptor macromolecules. For example, radioactive insulin and glucagon can bind with surprising specificity to inorganic substances such as talc, alumina powder,

and microsilica. An example is presented of specific insulin binding to talc which demonstrates a phenomenon which has been interpreted as "positive cooperativity" in studies of binding with biological tissues. Since this effect cannot be due to receptor interactions or true receptor cooperativity, it is suggested that this effect is due to hormone-hormone interaction and ligand-ligand association. The consequences of such processes for interpreting the results of binding studies are discussed. Such ligand self-aggregation, as well as nonspecific binding, can complicate Scatchard plots and give the impression of second-order binding sites.

Other problems discussed relate to the difficulty of ascertaining precise kinetic constants of binding when the receptor concentration in the assay medium is exceptionally high, as is usually the case in binding studies which utilize ligands (hormones) of insufficiently high specific activity. In addition, assessment of binding affinity on the basis of competition or displacement curves with native or unlabelled hormones will underestimate the true affinity constant. These pitfalls will be evident as well in Scatchard plot analyses. In addition, it is pointed out that certain hormones can cross-react with receptors for other sometimes closely related hormone receptors. In such cases the affinity of the interaction is usually lower than that of the hormone or its true receptor. Serious problems of interpretation can also result when binding data is obtained on mixed cell populations since the assumption is frequently made that all of the cells present are contributing equally to the binding process. The erroneous conclusion can be made, for example, that few receptors per cell exist in a given clinical state when in actuality the change is in the relative composition of the cells under study. The need for correlating the affinity of binding with the affinity for biological responses was discussed and the problems frequently encountered in such interpretations were described.

The possible mechanisms by which hormone-receptor complexes, once formed, can modulate membrane-localized functions were discussed. These mechanisms were illustrated with recent studies on the action of cholera toxin on cell membranes.

- - -

Biochemical Differences in Platelet Activation by Different Antigens

by Dr. Peter Henson - Scripps Clinical and Research Foundation,
La Jolla, California

Rabbit platelets are activated by different stimuli (collagen, platelet activating factor (PAF) from basophils, anti-platelet antibody, zymosan with complement bound to it (ZC) and thrombin) to secrete their preformed content of vasoactive amines - mediators of acute inflammatory reactions. The secretory event requires Ca++ and energy, and is inhibited by colchicine and agents which increase intracellular cAMP. During secretion, intracellular cAMP levels drop. Di-isopropylphosphofluoridate (DFP) also inhibits secretion, but only if present during the reaction of stimulus with cell. The stimulus-dependent activation of a precursor serine esterase on the platelets is suggested. Support for this hypothesis comes from observations of inhibition of secretion by small molecular weight esters. From inhibition data with series of phosphonate inhibitors and with different esters, it appears that each stimulus activates a unique serine esterase involved in secretion. It is postulated that this process represents an

important mechanism of cell activation.

- - -

The Generation and Release of Unstored Mediators by IgE-dependent Reactions

by Dr. K. Frank Austen - Harvard Medical School at Robert B. Brigham Hospital, Boston, Massachusetts

That human IgE mediates the immunologic release of histamine, slow reacting substance of anaphylaxis (SRS-A), and the eosinophil chemotactic factor of anaphylaxis (ECF-A) from human lung fragments has been established by showing deletion of activity with specific immunoadsorbents, competition for sensitization with IgE myeloma protein, and releasing activity with specific anti-IgE. The biochemical pathway initiated by interaction of IgE with specific antigen, appears to involve a DFP-sensitive esterase(E), anaerobic glycolysis, and certain cations, and is modulated by receptors of the adrenergic and cholinergic prototype. The chemical mediators presumably alter pulmonary function both by a direct action, perhaps particularly pertinent for the smaller peripheral airways, and by an effect on the epithelial irritant receptors to initiate a reflex cholinergic discharge with constriction of the larger, central airways and possibly further enhancement of mediator release.

- - -

The Terminal Events in the Release of Preformed Mediators by IgE-dependent Mechanisms

by Dr. Lawrence Lichtenstein - Johns Hopkins School of Medicine at Good Samaritan Hospital, Baltimore, Maryland

The preformed mediators discussed, which are released from human basophils by the antigen-IgE antibody interaction, are histamine, SRS-A, ECF-A, and a newly defined TAMe esterase which appears to be a kallikrein, since it generates kinin from human serum kininogen. Insofar as these have been studied, the mechanism of release of each is similar: the process is active, energy requiring, and controlled by hormone-receptor interactions which involve the cyclic AMP system. Histamine, acting through an H2 receptor, can feedback to inhibit the release of each of these mediators. The H2 receptor is also present on T lymphocytes and, in fact, seems to mediate a general anti-inflammatory role of histamine. The release process can be divided into two stages. The cyclic AMP-active agents inhibit in the first stage while agents which impair calcium flux, the generation of metabolic energy or microtubule aggregation act in the second stage. Each of these mediators is also released by the calcium ionophore A23187. This material appears to bypass the first stage of release. Studies of ionophore histamine release, together with the data mentioned above, allow us to propose a sequence of steps which leads to the release of the preformed mediators of anaphylaxis.

- - -

SESSION III: MEDIATORS OF T CELL - B CELL COOPERATION
Chairman - Dr. Richard W. Dutton

T Cell Mediator as a Maturation Signal in B Cell Response to Antigen

by Dr. Richard Dutton - University of California, San Diego, La Jolla, California

A brief review of the development of the concept of T cell-B cell interaction was presented. This included an account of the more recent developments in this field and showed that the originally simple model was rapidly becoming more complex. The four presentations in this session represent further examples of this increasing complexity. Our own studies have shown that the proliferation phase of the humoral immune response can be initiated in the complete absence of T cell helper activity. The T cell help, which is needed later in the response, supplies a maturation signal, which causes the differentiation of the already responding B cell into a mature antibody-secreting lymphocyte.

\- - -

Enhancing Factors for IgE and IgG Antibody Response

by Dr. Kimishige Ishizaka - Johns Hopkins University at Good Samaritan Hospital, Baltimore, Maryland

An in vitro culture system was set up to observe secondary anti-hapten IgE antibody response of rabbit lymph node cells. Rabbits were immunized with dinitrophenyl derivaties of either Ascaris extract or Ragweed extract Fr. D included in alum, and their mesenteric lymph node cells were stimulated with an appropriate antigen. For the anti-hapten IgE antibody response, both hapten specific precursors and carrier-specific helper cells are required. Helper cells for IgE antibody response were obtained when rabbits were immunized with the carrier included in alum. Immunization with the same carrier included in complete Freund's adjuvant raised carrier specific cells which have helper function for IgG antibody response but not for IgE antibody response. Helper function of carrier-specific cells was replaced by enhancing soluble factor which was obtained by stimulation of carrier-primed lymphocytes with free carrier. Anti-hapten antibody response was observed by stimulating DNP-primed cells with hapten-heterologous carrier conjugate followed by culture of the stimulated cells in cell free supernatant containing enhancing soluble factors. The soluble factors obtained in our system do not contain antigen, lack the immunoglobulin determinant and carrier-specificity. Evidence was obtained that enhancing factor for IgE antibody response is different from the factor for IgG antibody response. When donors of carrier-specific cells were immunized with the carrier included in alum, cell free supernatant obtained from the carrier-specific cells enhanced both IgE and IgG antibody responses. If the donors were immunized with the same carrier in complete Freunds adjuvant, cell free supernatant obtained from the carrier primed cells enhanced IgG but not IgE antibody response. Fractionation of cell free supernatant having both IgE and IgG enhancing activities indicated that enhancing activities for IgE and IgG are associated with different molecules.

As the process of antibody response can be divided into two stages: namely, activation of hapten-specific cells by hapten-heterologous carrier conjugate and differentiation (and proliferation) of activated cells to antibody forming cells in the presence of soluble factor, mechanisms of B cell activation was studied. It was found that divalent anti-immunoglobulin (Ig), but not the monovalent antibody fragment, activated hapten-primed IgG-B cells which formed anti-hapten antibody in the presence of non-specific enhancing factor. The results indicated that bridging of cell-surface immunoglobulin is an initial step of B cell activation. The activation of B cells by anti-Ig or hapten-heterologous carrier conjugate was enhanced by increasing intracellular cyclic AMP level. By contrast, the second stage of antibody response, which involved differentiation and proliferation of B cells and secretion of antibody, was inhibited by cyclic AMP.

- - -

Antigen Specific and Nonspecific Mediators of T Cell - B Cell Cooperation

by Drs. John W. Kappler and Philippa Marrack Hunter - University of Rochester, Rochester, New York

Evidence was presented for two types of helper T-cells in the mouse specific for a protein antigen, keyhole limpet hemocyanin (KLH). The first cell is able to help B-cells respond to the hapten, trinitrophenol (TNP), when coupled to KLH presumably through the participation of KLH-specific T-cell factors. The second helper cell when stimulated with KLH is able to help B-cells respond to red blood cell antigens, but not TNP-KLH, through the production of a non-antigen-specific factor.

Both types of helper cell were shown to be present in the spleens of KLH-primed mice, although they occurred with different frequencies. They were shown to be different T-cells by their segregation from each other *in vitro* at limiting dilution.

- - -

Cells and Factors Affecting T Cell Function

by Dr. Robert I. Mishell - University of California, Berkeley, California

Several examples of variability commonly encountered in experiments involving *in vitro* generation of immune responses were discussed. The hypothesis that much of this variability involves environmental effects on accessory cell functions was advanced. Experiments delineating the specific role of accessory cells and factors produced by them in protecting T cells from inactivation by hydrocortisone were presented in detail. Protection by *in vivo* activated "A" cells was shown and advanced to explain occasional examples of hydrocortisone resistance which appear in the literature. Finally a few other examples of accessory cells regulating T cell function were presented.

SESSION IV: MEDIATORS OF CELLULAR IMMUNITY - I
Chairman - Dr. Gale A. Granger

B Cell Lymphokine Production by Trypsin Derived C3b

by Dr. Ann L. Sandberg - Laboratory of Microbiology and Immunology
National Institute of Dental Research
National Institutes of Health

Trypsin cleavage of the third component of guinea pig complement (C3) (1% trypsin, pH = 7.5, 60 sec., 20° C.) yields a fragment which stimulates guinea pig lymphocytes to produce a chemotactic lymphokine. Under these digestion conditions the fragments obtained (C3a and C3b) are similar to those generated via immune activation of the complement sequence. In contrast, further digestion of C3 (2% trypsin, pH - 7.5, 1 hr., 20°C.) yields C3c and C3d which are inactive as inducers of this lymphokine. These digested C3 preparations contain only minimal inherent chemotactic properties. The C3 fragments, C3b and C3d, were characterized immunochemically and by their ability to inhibit rosette formation between lymphocytes and erythrocyte-antibody-C complexes bearing C3b or C3d. C3 and its digestion products were added to spleen cell cultures and 48 hr. supernatants were assayed for chemotactic activity in modified Boyden chambers. Chemotactic activity was quantitated by microscopic enumeration of guinea pig macrophages which had migrated through 5 u polycarbonate filters in response to the cellular derived factor. The lymphocyte production of chemotactic factor was completely abrogated by the adsorption of the C3b containing digest with goat anti-guinea pig C3 coupled to Sepharose 4B prior to its addition to spleen cell cultures. The active C3 fragment was C3b as demonstrated by Sephadex G-75 column chromatography of the 60 sec. digestion products of C3. The lymphocyte stimulating activity eluted only in the exclusion volume (MV $>$ 50,000 daltons). B (bone marrow derived) cell populations ($>$ 95% B cells) obtained by removal of rabbit erythrocyte rosetted T (thymus derived) cells produced this chemotactic factor when stimulated with C3b containing preparations. In contrast, T cells obtained by double passage of spleen cells through nylon wool columns ($>$ 95% T cells) were incapable of elaborating this lymphokine when stimulated with C3 fragments but did respond to phytohemagglutinin. These studies imply that free C3b generated by proteolytic enzymes may contribute to immunological sequelae by virtue of its ability to generate lymphokines.

- - -

Interferon and The Immune System

by Dr. Thomas C. Merigan - Stanford University Medical School,
Stanford, California

Recently, interferon has been associated with the immune response in two ways. First, interferon has been noted as a product of the cell-mediated immune response associated with blastogenesis and the production of other mediators. Secondly, interferon has been observed to have both immunodepressing and immuno-enhancing effects in lymphoid cell culture systems.

Observations in our laboratory have documented the ability of human lymphocyte preparations in the presence of macrophages to produce interferon on an immune specific basis to undergo blastogenesis in response to PHA, PPD, vaccinia or herpes simplex antigen. In the case of the latter stimulus, the T-lymphocyte has been shown to be the source of the interferon production

and carry the memory for the interferon response related to recent prior herpes simplex infection. Whereas, blastogenesis appears to be related even to remote prior infection.

Our studies have demonstrated large doses of interferon inhibiting the subsequent production of circulating antibody to sheep red blood cells as well as antibody forming cell production in the spleen. Furthermore, if interferon is given immediately after antigen, immuno-enhancement of circulating antibody levels to sheep red blood cells is observed. The immunosuppressive effect can be observed with Salmonella typhimurium polysaccharide antibody production as well and, in this latter case the inhibition seems to be acting through an effect on the B-lymphocyte when the mechanism is studied in vitro.

- - -

LT Secretion, Regulation and Its Action On Target Cells

by Dr. Gale A. Granger - University of California, Irvine, California

Studies were conducted to examine if there are control mechanism(s) regulating lymphokines released by mitogen-activated human lymphocytes in vitro. These studies were made possible by our development of an in vitro assay which quantitates the amount of lymphotoxin secreted into a supernatant medium. We found evidence of control mechanisms operative at several levels:

(A) Initiation
(B) LT secretion is an inducible event, can be turned on and off, and only occurs when the secreting cell is in contact with the stumulating agent.
(C) The levels reached in both mitogen and antigen MLC stimulated cultures are under some form of feedback control.
(D) There is a sequential appearance in MLC reactions of allogeneic factor during the first three days of culture, followed by the rapid rise in LT as a suppressive factor.

These studies begin to reveal that lymphokines are under stringent control systems. These control systems may explain how soluble nonspecific molecules can be important effectors in cell-mediated immunity.

- - -

Relationship of Soluble Lymphoid Cell Produced-toxins to Cell-mediated Cytotoxicity

by Dr. Zoltan J. Lucas - Stanford University Medical Center, Stanford, California

Human lymphocytes activated by phytohemagglutinin release several cytotoxins into the supernatant fluids. Toxins are assayed by lysis of mouse L cells growth-inhibited by actinomycin D. One toxin (called Early Lymphotoxin, ELT), detected within 4 hours after activation, is maximally detected at 24 hours, whereafter no further net increase occurs. Another toxin (called Late Lymphotoxin, LLT), is initially detected 24 - 36 hours after activation; its rate of release into the medium progressively increases for

72 to 96 hours. ELT and LLT are also distinguishable by different volumes of distribution in Sephadex G-100 chromatography (ELT, MW 45,000 daltons; LLT, MW 80,000 daltons) and by inhibition of LLT by rabbit anti-human LLT, which does not inhibit ELT. A third toxin present in lymphoid tissues like the spleen, termed Adherent Cell Toxin (ACT), is elaborated by plastic-adherent cells, presumably macrophages, immediately upon attachment of the cells to a solid surface. Its release does not require mitogen-stimulation. Its volume of distribution on Sephadex G-100 chromatography is similar to that of ELT (MW, 45,000 daltons), but these can be separated by subsequent DEAE chromatography. It is suggested that, because of its time of release, ELT functions as a cytotoxin, whereas LLT may be a lymphocyte regulator.

SESSION V: MEDIATORS OF CELLULAR IMMUNITY - II
Chairman - Dr. John R. David

Lymphocyte Mediators and The Activation of Macrophages

by Dr. John R. David - Harvard Medical School
Boston, Massachusetts

For many years, it has been apparent that macrophages play an important part in resistance to many infections. These cells ingest and dispose of a variety of organisms: when obtained from recently immunized animals, they exhibit an enhanced ability to do this and have been termed "activated". More recent studies suggest that in vivo immune activation of macrophages requires the interaction of specifically sensitized T lymphocytes with the appropriate antigenic agent. Once activated, the macrophages may exhibit a certain degree of nonspecific antimicrobial activity.

How does the interaction of lymphocytes with antigen lead to the activation of macrophages? The events that occur in vitro are not known. In vitro studies on the mechanisms of cellular hypersensitivity and immunity, however, have shown that such stimulated lymphocytes produce a number of soluble mediators, several of which affect macrophages.

A number of laboratories have shown that macrophages incubated with lymphocyte mediators show altered morphology, metabolism and function. Of especial interest are the changes that involve the macrophage membrane. Macrophages incubated in MIF-rich Sephadex fractions exhibit increased adherence to glass and plastic, increased ruffled membrane movement, increased phagocytosis of some particles such as dead mycobacteria, but decrease in others such as aggregated hemoglobin, enhanced pinocytosis and increase in incorporation of glucosamine and decrease in electron dense surface substance. There is an increase in the activity of the membrane associated enzyme - adenylate cyclase. Other changes include increased glucose oxidation through the hexose monophosphate shunt and increased number of cytoplasmic granules when assessed morphologically. Recently, such activated macrophages have been shown to produce collagenase. Of special interest is that finding that such macrophages activated by lymphocyte mediator exhibit bacteriostasis to bacteria such as Listeria and enhanced ability to kill syngeneic tumor cells. Clearly, lymphocyte mediators can activate macrophages. It is quite possible that other factors are also involved including direct lymphocyte-macrophage interactions.

- - -

Interaction of MIF With The Macrophage

by Dr. Heinz G. Remold - Harvard Medical School, Robert B. Brigham Hospital, Boston, Massachusetts

Little is known how lymphocyte mediators, in special MIF, interact with the macrophage. In order to investigate this problem, studies were undertaken which deal with the following two subjects: (1) L-fucose on the MIF receptor of the macrophage, and (2) the enhancement of MIF activity by plasma esterase inhibitors. It could be shown that incubation of macrophages with L-fucose abolishes the activity of guinea pig migration inhibitory factor on the macrophages. Other sugars, such as D-glucose, D-galactose, L- rhamnose, methyl-D-mannoside, and N-acetyl-D-glucosamine, had no effect. The abolition of MIF activity by L-fucose was reversible. When macrophages were incubated with L-fucosidase, a glycosidase which hydrolyzes terminal L-fucose from oligosaccharides of the cell surface, the macrophage no longer responded to MIF. On the other hand, MIF incubated with L-fucosidase was still active. This experiment strongly suggests that L-fucose is an essential part of a macrophage receptor for migration inhibitory factor.

Secondly, we studied the effect of plasma esterase inhibitors on the inhibition of migration of guinea pig peritoneal macrophages caused by MIF, because cell-associated esterases have been shown to play an important part in a number of regulating processes. Reincubation of macrophages with the plasma esterase inhibitors α_1-antitrypsin, C1 esterase inhibitor, and antithrombin heparin cofactor enhances the inhibition of migration of these cells caused by MIF. Heparin prevents the MIF-enhancing effect of antithrombin-heparin cofactor. This effect was shown to be specific for antithrombin-heparin cofactor, probably due to its ability to bind heparin. Modification of arginine residues of antithrombin-heparin cofactor and soy bean trypsin inhibitor, by 2,3-butanedione, does not alter their MIF-enhancing capacity, whereas the thrombin and trypsin inhibitory activity is ablated. These findings indicate that modification of the arginine residues in antithrombin-heparin cofactor and soy bean trypsin inhibitor has no effect on the interaction of these inhibitors with the macrophage, suggesting different binding sites.

\- - -

Regulation of Chemotaxis and Other Lymphokine Mediators

by Dr. Peter A. Ward - University of Connecticut, Farmington, Connecticut

Normal human serum contains a chemotactic factor inactivator (CFI) which irreversibly inactivates the complement derived chemotactic factors (C3 and C5 fragments and C567). CFI also inactivates the monocyte chemotactic activity in preparations of the C5 fragment as well as the activity present in culture fluids of antigen-stimulated lymphoid cells. Incubation of CFI-rich preparations also leads to inactivation of migration inhibition factor (MIF) present in culture fluids of antigen-stimulated human lymphoid cells. The inactivation of MIF appears to be irreversible. In addition, the MIF inactivator is heat-labile; prior heating of CFI-rich preparations at 56°C for 1 hr. abolishes the inactivation of MIF. Recently CFI has been fractionated into two forms, an α globulin and a β globulin. Both forms

have the ability to inactivate MIF. These results indicate that there is present in human serum a material that inactivates complement-derived and lymphocyte-derived mediators. (Supported by NIH Grants AI 09651, AI 11526, AI 12225).

- - -

The effects of An Invertebrate Macrophage Product on the Vertebrate

by Dr. Robert A. Prendergast - Johns Hopkins School of Medicine, Baltimore, Maryland

Delayed inflammatory skin reactions, inhibition of macrophage migration, and activation of mammalian peritoneal exudate macrophages with destruction of a target cell monolayer are caused by a protein of approximately 32,000 MW, isolated from coelomocytes of the sea star Asterias forbesi (SSF). These responses are similar to those induced by the supernatant of immune vertebrate T cells following incubation with specific antigen. The direct role of SSF on vertebrate lymphocytes was assessed in vivo and demonstrated that injection of 1 mg SSF plus P815 mastocytoma cells into the peritoneal cavity of C38/black recipient completely abrogated the generation of cytolytic T cells. Further, in vitro treatment of spleen cells from syngeneic donors with SSF abrogated the development of a primary antibody response to sheep red blood cells in recipients following concomitant immunization. In contrast, similar treatment of cells from a primed donor did not affect the secondary response in the recipient after challenge with sheep red blood cells. The effect of SSF in cell-mediated resistance to Listeria infection was also investigated, and demonstrated that SSF-treated mice became highly susceptible to infection with a normally sublethal dose of this organism. Treatment of immune mice with SSF did not alter their immune status. Treatment of mouse spleen cells or human peripheral blood lymphocytes with SSF in vitro demonstrated marked suppression of tritiated thymidine incorporation induced by exposure to the T cell mitogens, phytohemagglutinin, or concanavalin A, and, in addition, suppressed response in a mixed lymphocyte culture. Viability of the cells whose division was suppressed by SSF was demonstrated to be normal following prolonged incubation by trypan blue exclusion, spontaneous rosette formation of human peripheral blood lymphocytes and sheep red blood cells, the ability of "killer" T cells to lyse allogeneic target cells, and the ability of mouse spleen cells to form antibody in culture in the Mishell-Dutton system if SSF is withheld during the first 24 hours following exposure to antigen.

These data indicate that high doses of SSF have a marked inhibitory effect on generation of a primary immune response to T-dependent antigens, but have no such effect on a previously immune cell population in demonstration of its normal competence.

- - -