The Fifteenth Midwinter Conference of Immunologists was held January 24-27, 1976, at Asilomar Conference Grounds, Pacific Grove, California, with Leonard A. Herzenberg and Hugh O. McDevitt serving as co-chairmen. The subject of the Conference "Lymphocyte Differentiation" was discussed during five half-day sessions by invited speakers. One afternoon of the Conference was devoted to workshop programs, consisting of eight working sessions and three panel discussions held concurrently. This format permitted smaller groups to meet and allowed for unscheduled presentation of individual data. The workshop programs were organized by Leonore A. Herzenberg and Eli Sercarz.

The second annual Dan H. Campbell Memorial Lecture was given by George D. Snell, Emeritus, The Jackson Laboratory, Bar Harbor, Maine. The lecture was preceded with introductory remarks by George Feigen (Stanford University), a close associate and friend of Dr. Campbell's for many years. He shared with the audience some of his memories of the colorful personality that was Dan Campbell, of his many achievements, and of his deep and abiding love of immunology. Ray Owen (California Institute of Technology) introduced Dr. Snell, whose topic for the evening was cell surface alloantigens of lymphocytes.

Serologically defined (SD) alloantigens of murine lymphocytes now number 18. Six of these are determined by the H-2 complex. Transplantation techniques have revealed 45 histocompatibility (H) alloantigens, some and perhaps all of which probably are represented on lymphocytes, as well as on skin. Serological demonstration of H alloantigens has proved difficult. Only H-2K, H-2D, and perhaps Ly-4 represent unmistakable overlaps between

the SD and H groups. Except for H-2K and H-2D, the SD alloantigens generally show a narrow tissue distribution. The H antigens, on the contrary, seem to show a wide tissue distribution. Dr. Snell suggested that a possible function of SD alloantigens is the regulation of cell interactions. H antigens serve some other function, possibly transport. Most cell interactions and cell migrations, such as those now well documented for lymphocytes, are probably highly specific for specific cell types. The cell surface components which regulate them should therefore be limited in their distribution. If H-2K and H-2D are cell interaction substances, they must regulate interactions of a much more general nature. Three methods by which SD alloantigen may regulate cell interactions are dimer formation, the interaction of complementary molecules, and the interaction of structured islands of membrane proteins with one another or with single cell surface products.

The first session of the Conference, convened by Irving Weissman (Stanford University School of Medicine), dealt with immunological differentiation of T cell differentiation. Ross S. Basch (New York School of Medicine) the first speaker, discussed prothymocytes, hemopoietic thymocyte precursors.

There exists in the hemopoietic tissues, a population of cells capable of restoring the lymphoid cell compartment of the thymus of an irradiated mouse. While the ultimate progenitor of this population seems to be the pluripotential stem cell (CFU_s) of the bone marrow and spleen, neither the origins nor the state of differentiation of the thymus precursor or "prothymocyte" have been firmly established. Indirect evidence indicates

that the prothymocyte is a specialized cell, committed to thymocyte differentiation and discrete from the precursors of B lymphocytes.

Dr. Basch and his associates have developed a quantitative assay for this cell based upon its ability to repopulate the thymus of irradiated mice and have used this to examine the properties of this stem cell population. Prothymocytes are found amongst the "null" cells of both bone marrow and spleen. They are 6-8 fold more frequent in bone marrow than the spleen and are absent from peripheral lymph nodes. Large numbers are present in neonatal animals. These cells are relatively cortisone resistant and radioresistant. They lack detectable surface immunoglobulins and Thy-1 alloantigens. They do bear on their surface several antigens reactive with rabbit anti-mouse brain antiserum and heterologous anti-thymocyte sera. These cells contain terminal deoxyribonucleotidyl transferase, an enzyme characteristically found in cortical thymocytes.

Treatment of these cells with purified peptide thymopoietin or a variety of non-specific inducing agents results in the rapid expression of thymocyte differentiation antigens on the cell surface and an alteration in response to plant lectins.

No evidence of specific antigen recognition by these cells has yet been adduced.

David Baltimore (Massachusetts Institute of Technology) next described two different assays used in his laboratory to define different populations of immature lymphocytes.

One assay is for the enzyme terminal deoxynucleotidyl transferase (TdT), the other assay is for cells transformable *in vitro* by Abelson mouse

leukemia virus (A-MuLV). TdT is found in most thymocytes as well as in bone marrow cells but is absent in peripheral lymphocytes. In marrow, much of it occurs in a population of cells that can be induced by thymopoeitin to express a thymic antigen. A-MuLV transforms cells of the B-cell series *in vivo*. *In vitro* the cells transformed are very immature and contain a trace of TdT--they may be cells that are not committed to either the B- or T- cell pathway.

In the presentation that followed on viral genes and differentiation antigens on thymus and spleen, Richard A. Lerner (Scripps Clinic and Research Foundation) stated that, in mice expression of the endogenous oncornaviral genome is under differentiation control. Thus, expression of virion proteins can be studied as any other inherited differentiation antigen. The major oncornaviral envelope glycoprotein, gp70, is coded by the viral genome and is a component of the surface of T-cells and sperm. The G_{IX} alloantigen is now known to be a type specific determinant of the gp70 molecule and the genetics of the G_{IX} alloantigen are, thus, applicable to gp70. G_{IX} gp70 is present on T-cells and sperm of 129 G_{IX}^{+} and $C_{57}B_6G_{IX}^{+}$ but not the congenic 129 G_{IX}^{-} and $C_{57}B_6G_{IX}^{-}$ mice. In addition to expression on the surface of cells following certain pathways of differentiation, gp70 has a characteristic pattern of expression during embryogenesis and is a prominent component of the surface of cells in the 14-day hematopoietic liver. Of all the oncofetal-differentiation antigens yet reported, gp70 has the advantage that both it and its mRNA are available in pure form. Dr. Lerner expects this protein to play a prominent role in studies of induction of gene expression leading to the T-cell phenotype.

The first session concluded with Robert Hyman (The Salk Institute of Biological Studies), who spoke on somatic genetic studies on cell surface antigen variants.

The objective of such studies is to understand the genetic mechanisms by which cells regulate their expression of surface antigens. Somatic genetic analysis of variants of tumors of murine lymphoid cells is used to enumerate the structural and regulatory genes for several model surface antigens and to study the mechanisms which regulate their expression.

Four independent variants which lack detectable Thy-1 antigen have been isolated from cloned murine lymphomas growing in tissue culture. The basis of the variation is genetic. The four variants can be grouped into three complementary classes; the variant phenotype is invariably recessive when variant cells are fused with parental lines; fusions of complementary variants derived from cells expressing alternative alleles of the Thy-1 antigen express both alleles. Biochemical analysis indicates that the 25,000 molecular weight glycoprotein bearing the Thy-1 determinant is not present on the surface of the variant cell lines. At least one class of variants synthesizes an altered glycoprotein molecule. These results are consistent with a model in which the variants are presumed to represent mutations in genes coding for glycosyltransferases responsible for attachment of sugars to the glycoprotein bearing the Thy-1 determinant. The allelic specificities may be due to differences in terminal sugars or to alterations of the protein backbone.

A variant line which lacks detectable TL antigen has also been isolated. This variant has no detectable serologically defined H-2 antigens but has

parental amounts of Thy-1.2. Cell hybridization experiments indicate that the structural genes coding for both TL and the antigens of the H-2 complex are present in the variant. Karyotypic analysis shows that one chromosome has been lost from the variant cell line.

The second session on differentiated functions of T cells, with Leonard A. Herzenberg (Stanford University School of Medicine) as chairman, opened with Harvey Cantor (Harvard Medical School) who discussed the generation of functional T cell subclasses.

Two T cell subclasses, which are products of separate lines of differentiation were described. Cells of the Lyl subclass generate helper activity in both primary and secondary responses to SRBC. In contrast, after priming with SRBC, cells of the $Ly2^{+}$ subclasses, in particular Ly23 cells, have suppressive activity. The degree of Ly23-mediated suppression is directly proportional to the amount of antigen (SRBC) used for priming. Suppression by Ly23 cells is specific because Ly23 cells from SRBC-primed animals do not suppress the response to HRBC, and vice versa. Thus, both cytotoxic and specific suppressor functions are mediated by T cells of a subclass, provisionally designated T_{CS}, which can be distinguished from helper T cells (T_H), by their Ly phenotypes. It remains to be determined whether killing and suppression are functionally interrelated properties of a single Ly23 subclass, or whether the Ly23 population comprises two subclasses whose surface phenotypes are not yet distinguishable by immunogenetic criteria.

The second speaker, Robert D. Stout (Stanford University School of Medicine) pointed out in his presentation on the Fc receptor on thymus

derived lymphocytes that a high degree of functional heterogeneity exists within the thymus-derived (T) lymphocyte class.

T lymphocytes have been shown to initiate graft-vs-host reactions, generate proliferative and cytotoxic responses to alloantigen, and to exert amplifier, helper, and suppressor effects on both antibody production and cellular immunity. A large number of cytotoxic and fluorescent probes reactive with T cell membrane components have been prepared in attempts to delineate T cell subpopulations and their maturational stages. They have used, as such a probe, fluoresceinated antigen-antibody complexes (F*AgAb), to define two distinct subpopulations of peripheral T cells--Fc receptor positive (Fc^+) and Fc receptor negative (Fc^-) T cells. Functional studies on populations of Fc^- and Fc^+ T cells, purified on the fluorescence-activated cell sorter (FACS), revealed that the Fc^- T cell subset was not responsive to concanavalin A (Con A), and contained both the helper T cells involved in collaboration with B cells in generation of humoral immune responses and the precursors of cytotoxic lymphocytes generated in the cell-mediated lymphocyte (CML) response to H-2 allogeneic cells. In contrast, the Fc^+ T cell subset was responsive to Con A, did not contain helper T cell activity, but did contain both the differentiated cytotoxic lymphocytes (CL) and the amplifier T cell involved in amplification of CML responses.

Arther Kimura (University of Uppsala) reported on studies, by himself and his associates, Leif Andersson and Hans Wigzell, that analysed the function of membrane determinants on cytotoxic T lymphocytes.

A variety of antisera have been used against effector T lymphocytes in attempts at interfering with specific recognition and lytic activity of

these cells. The results of these studies have proven to have little, if any, impact on T cell-mediated killing in the absence of complement. One approach to the study of killer relevant structures on effector T cells has been by the production of heteroantisera in rabbits against purified populations of both mouse and rat effector T lymphocytes. These sera have been consistently powerful in inhibiting effector T cells in pretreatment, washout assays not involving complement. The blocking capacities of these sera have been interpreted as the presence of reactive antibody against killer relevant structures present on the effector T cell membrane. The membrane structure(s) have been further defined by a gel precipitation reaction against NP-40 solubilized membrane preparations of cytotoxic T lymphocytes. The relevance of the mouse membrane precipitate was established by making a second antisera in rabbits against the mouse component of the precipitin line. Such sera was tested and found to be efficient in blocking killer T cell activity in a dose-dependent manner.

Another membrane structure found on a proportion of activated killer T cells which is currently of considerable interest is the Fc receptor. Although unnecessary for "classical" T cell-mediated killing as measured *in vitro*, the Fc positive killer T cells can be shown to function quite efficiently in the killing of antibody coated target cells whereas Fc negative killer T cells are not. This type of T cell-mediated killing may represent an efficient pathway for target cell damage under conditions that would not favor the more "classical" type of T cell killing.

Separation of helper and suppressor T lymphocytes on a ficoll velocity sedimentation gradients was next described by Richard Dutton (University of California at San Diego) from studies by Harley Tse and himself.

A 5-20% Ficoll velocity sedimentation gradient was successfully used to separate Con A-induced helper and suppressor T cells. When titrated into a constant number of fresh normal spleen cells responding to SRBC, cells from the top pool show stimulatory effects while those from the bottom pool show inhibitory activity. Both activities were found to be Con A dependent and anti-θ sensitive. They concluded that Con A-induced helper and suppressor T cells are distinct subpopulations. Such separation will allow further characterization of these cell types.

The last speaker in the second session was William E. Paul (National Institute of Allergy and Infectious Diseases). He spoke on the genetic control of T lymphocyte activation.

Immune responses to a series of specific antigens are controlled by a group of immune response (Ir) genes which are encoded within the major histocompatibility complex. Since all responses controlled by Ir genes are dependent on the function of T lymphocytes, it has been postulated that a major site of expression of Ir genes is within the T lymphocytes. Unfortunately, it has been difficult to study this directly in mice as a simple antigen-specific T lymphocyte proliferation assay has not been readily available.

Recently, such an assay has been developed based on the use of T lymphocytes purified from induced peritoneal exudates in immunized mice. Using this procedure, Paul and his associates studied the responsiveness of a series of mouse strains representing a large number of distinct MHC haplotypes in response to eight individual antigens, responsiveness to which is controlled by MHC-linked Ir genes. In each case, the responder status

previously assigned by antibody responsiveness was confirmed by the T lymphocyte proliferation assay. This indicates that none of the Ir genes under study can be exclusively expressed in B lymphocytes.

Alloantisera directed at gene products of the I-region of the mouse MHC were found to be powerful inhibitors of T lymphocyte activation by specific antigen. Such sera blocked activation in Ir gene controlled responses in both a "region-specific" and "haplotype-specific" manner indicating a close phenotypic relationship between Ia antigen and Ir gene products. A model in which these two gene products are represented as separate domains of a single polypeptide was proposed. It is envisioned that these domains are the products of separate, but linked genes joined by a translocation mechanism akin to that involved in the synthesis of immunoglobulin H and L chains.

The subject of the third session, with Hugh O. McDevitt (Stanford University School of Medicine) the chairman, was the 17th chromosome and cell recognition.

Rolf M. Zinkernagel and Peter C. Doherty (Scripps Clinic and Research Foundation and the Wistar Institute) presented data on H-2 compatibility requirements for virus specific T cell-mediated cytolysis: the altered self hypothesis.

For virus-specific cytotoxic T cells to lyse infected target cells, both must be compatible at the K or D but not the I regions of the H-2 gene complex. A similar constraint operates *in vivo* as demonstrated in adoptive transfer experiments. These findings suggest that effector T cells may be specific for virally altered self markers coded in K or D. This

conclusion is based on circumstantial evidence derived from experiments including cold target competitive inhibition *in vitro*, selective proliferation *in vivo* with F_1 hybrid and H-2 recombinant mice and from cytotoxicity experiments with H-2 mutant mice. Also, alloantigen reactivity could be easily explained within this model.

Cytotoxic T cells seem to be concerned mainly with immunosurveillance of self-markers coded in K or D and are different from noncytolytic helper T cells (and at least some DTH effector T cells), which may be sensitized to altered Ia or structures coded in the I region of H-2.

An alternative explanation for this restriction of effector T cells by the H-2 gene complex proposes the need for two interactions, an immunologically specific recognition plus a "physiological" self-self interaction. To accommodate the experiment findings, this model requires complex genetic mechanisms that regulate the phenotypic expression of such physiological interaction structures but its existance cannot be excluded as yet.

Next studies on cell mediated lympholysis of chemically modified autologous cells by Gene M. Shearer, Anne Marie Schmitt-Verhulst and Terry G. Rehn (The National Cancer Institute) were given by Gene M. Shearer.

Thymus-derived splenic lymphocytes from normal mice were sensitized *in vitro* to trinitrophenyl-(TNP) or N-(3-nitro-4-hydroxy-5-iodophenylacetyl-) β-alanylglycylglycyl-modified autologous spleen cells. Cytotoxic effector cells were generated which specifically lysed only ^{51}Cr-labelled modified spleen target cells treated with the same agent used for sensitization and which shared H-2K and/or H-2D alleles with the cells of the sensitizing phase. These results are compatible with either the intimacy or altered

self models. Experiments using F_1 hybrid responding lymphocytes sensitized and assayed with TNP-modified parental cells indicated the H-2 homology required for lympholysis was either between modified stimulating and modified target cells (altered self) or between responding and stimulating cells during the sensitization phase and between effector and target cells during the lytic phase (a two-stage intimacy model). Results of blocking experiments using anti-H-2 sera and anti-TNP antibodies indicated that the effector cells are directed against specificities associated with serologically detectable region products, and that some TNP groups are very close to H-2K and H-2D region products. Dominant, H-2-linked Ir genes were detected in the responding cell populations. Expression of these genes was necessary for generation of cytotoxic responses to altered self. Thus, the generation of a cellular immune response in this type of system is dependent on the modifying agent, the major histocompatibility complex product altered, and expression of the relevant Ir genes necessary for reactivity.

Michael Bevan (Salk Institute for Biological Studies) addressed the question "How are the products of major and minor histocompatibility expressed at the cell surface?".

Murine cytotoxic effector T cells can be generated by immunization with minor H antigens following an allogeneic immunization when responder and stimulator bear identical major H (H-2) genes. As in the viral and hapten modified systems, only targets which bear the same H-2 alleles are susceptible to lysis by such effector cells.

Specificity in the cytotoxic assay for the immunizing minor H allele has been shown using Snell's minor H congenic mice the H-7 pair, the H-8

pair and the H-3, H-13 pair. *In vivo* priming for a secondary cytotoxic response is also specific for the minor allele B10.LP ($H\text{-}2^b$, $H\text{-}3^b$, $H\text{-}13^b$). Mice injected with B10 ($H\text{-}2^b$, $H\text{-}3^a$, $H\text{-}13^a$) cells are primed for a response to B10 but are not primed for a response to LP/J($H\text{-}2^b$, $H\text{-}3^b$, $H\text{-}13^b$). Cytotoxicity is specific for the H-2 alleles also but H-2 different cells do cross-prime for a response to the same minor allele on different H-2 backgrounds. Dr. Bevan stated that (1) it is impossible that hundreds of minor H genes modify the 45,000 molecular weight K and D antigens as suggested in the altered self hypothesis; (2) if H-2 products modify minor H gene products as suggested, then the immune system still detects some cross-reaction between the same minor H antigen in association with different H-2 alleles.

The third session terminated with Karen Artzt (Cornell University Medical College) who spoke on serological detection of cross-reacting antigens specified by 3T/t complex haplotypes.

Antisera raised against three kinds of mutant T/t-complex sperm were rendered specific for the t^0, t^{w2} and t^9 haplotypes by absorption with T/+ sperm. Absorption analyses with these three cross-reacting reagents showed each haplotype to be complex consisting of both shared and unique specificities. A minimum number of specificities defined by these three haplotypes is six. Moreover, the unique specificities for t^0 and t^{w2} are lost when these chromosomes undergo exceptional recombinational events indicating there are at least two serologically detectable genes at the T/t complex separable by recombination. This, together with the serological complexity, supports the hypothesis that the T/t-complex may be an evolutionarily old, embryonic analogue of the adult MHC.

Marian Koshland (University of California at Berkeley) convened the 4th session on early events in B cell differentiation. The first speaker

was Samuel Black who discussed studies by G. J. Hammerling, C. Berek, K. Eichmann, K. Rajewsky and himself on idiotypes on B and T lymphocytes.

Using two separate idiotype systems they found that B and T lymphocytes with specificity for Group A Streptococcal carbohydrate (A.CHo) share the same idiotype on their antigen receptor sites. Furthermore, cells expressing these idiotypes can be specifically primed by exposure to anti-idiotypic sera.

Guinea pig anti-idiotypic sera were raised against idiotypes expressed on the A5A antibody clone of A/J mice and S117 antibody clone of BALB/c mice. A5A and S117 antibodies are specific for n-acetyl glucosamine the major antigenic determinant of A.CHo. The anti-idiotypic sera were fractionated in the IgG_1 and IgG_2 classes. Passive administration of anti-A5A idiotype sera of the IgG_1 class to mice which express the A5A idiotype (e.g., A/J, DBA/2, RF and ASW) primed both B and T cells of these animals so that they could generate a secondary immune response when challenged with Streptococcal A. type vaccine (Strep. A). Passive administrative of anti-S117 idiotype sera of the IgG_1 class to mice expressing the S117 idiotype (e.g., BALB/c, RD, DBA/2, 129 and BAB 14) also primed A.CHo specific B and T cells to give a secondary response on challenge with Strep. A. Strains of mice not expressing the A5A idiotype could not be primed with anti-A5A idiotypic sera and mice which did not express the S117 idiotype were not primed by exposure to anti-S117 idiotypic sera. Extensive *in vitro* analysis demonstrated that the anti-idiotype primed B and T cells responding to Strep. A. challenge were A.CHo specific and carried the appropriate idiotype. These experiments strongly suggest that B and T cells recognize antigen in the same fashion, i.e., by the products of Ig variable region genes.

In contrast pretreatment of A/J mice with guinea pig anti-A5A idiotype sera of the IgG_2 class resulted in the generation of a population of T cells which could suppress A5A positive antibody responses. These suppressor T cells expressed Ia antigens and could be trapped on a histamine column.

Allotype specific cooperator T cells and suppressor T cells was subject of the next talk by Leonore A. Herzenberg (Stanford University School of Medicine).

T lymphocytes exert opposing types of regulation of antibody responses. Cooperator (helper) T cells (Tc) facilitate the differentiation and expansion of B lymphocytes to antibody producing cells. Tc are essential for IgG production. Suppressor T cells (Ts) on the other hand, reduce primary and secondary antibody responses to specific antigens, antibody production in a specific class of immunoglobulins and total production of immunoglobulins carrying one of the parental allotypes in an allotypically heterozygous mouse. Since in most of these cases the suppressed response is T dependent, the net effect of T cell suppression is essentially the negation of T cell cooperation.

The antagonism between suppressor and cooperator T cell activities is directly demonstrable in adoptive transfer assays with allotype (Ig-1b) suppressor T cells found in allotypically heterozygous SJL x BALB/c mice (Ig^b/Ig^a) exposed perinatally to antibody to Ig-1b immunoglobulins. When co-transferred with carrier-primed Tc from non-suppressed mice and dinitrophenyl (DNP) hapten-primed B memory cells, the allotype Ts specifically suppress the appearance of cells producing Ig-1b antibody to DNP (Ig-1b

DNP-PFC). This suppression is reversed by increasing Tc; however, as Ts is increased, proportionally more Tc are required to achieve the same degree of reversal. Thus, the quantitative Ig-1b DNP-PFC response is controlled by the relative activity (proportional to number) of Ts and Tc transferred, rather than by the absolute amount of either.

This can be expressed as: Ig-1b PFC/B-cell = $k(Tc - \alpha \cdot Ts)$, where the constants k and α are empirically determined. This shows an excellent fit with the observed responses. Since this suppression is specific for Ig-1b, this data implies allotype specific Tc.

In recent experiments she and her associates have found:

1) That Ig-1b memory B cells in SJL x BALB/c hybrids require Ig-1b specific Tc. The Tc are not present in carrier-primed mice congenic to SJL x BALB/c but homozygous for Ig^a;
2) That *in vitro* pretreatment of spleen cells from carrier-primed non-suppressed SJL x BALB/c donors with soluble "factors", produced by cultured spleen cells from Ig-1b suppressed mice, selectively removes Ig-1b Tc;
3) That spleen cells from carrier-primed suppressed mice have no Ig-1b Tc in adoptive transfer when tested after removal of Tc either by size separation or cytotoxic treatment with anti Ly-2 and complement; and
4) That hapten-primed suppressed mice have normal numbers of functional Ig-1b memory cells.

Thus, she concluded that suppression of Ig-1b production by allotype suppressor T cells results from removal of specific Tc which help in Ig-1b memory expression.

Changing patterns of immunoglobulin receptors during B lymphocyte differentiation were described by Ellen S. Vitetta (University of Texas Southwestern Medical School).

Recent studies from her laboratory have shown that murine B lymphocytes express two classes of cell surface immunoglobulin, IgM and an IgD-like molecule. The latter is identified by its structural similarities with human IgD and its predominance on the cell surface. The IgD analog is synthesized and slowly turned over by the cells on which it resides and precedes IgM during both ontogeny and differentiation of B cells. Biochemical and immunofluorescence studies have establsihed that three classes of Ig-bearing B cells exist; those with only IgM or IgD and those expressing both isotypes. In the spleen, approximately 20% of the B cells express only IgM, 30-40% express only IgD and the remainder bear both isotypes. In contrast, in the lymph nodes and Peyer's patches, 60-70% of the B cells express only IgD and 10% only IgM. During ontogeny it appears that IgM-only cells give rise to double bearers and then to IgD-only cells.

Recent experiments demonstrate that neonatal B cells, which are predominantly IgM-only cells are tolerized with 0.1% of the tolerogen requied to tolerize adult spleen cells. After stimulation of adult B cells with LPS, IgM bearing cells (μ-only or $\mu + \delta$) give rise to IgM secreting plasma cells. In contrast, IgD-only cells proliferate but do not secrete IgM. It was therefore suggested that IgD-only cells will eventually give rise to IgG bearing lymphocytes and/or IgG secreting plasma cells.

This session concluded with Irwin Scher's talk on work done in collaboration with A. Ahmed, R. Wistar, S. O. Sharrow and W. E. Paul (Naval Medical

Research Institute and National Institutes of Health) delineating characteristics of subpopulations of B lyphocytes.

They have analyzed the maturation of B lymphocytes in the mouse by studying the density of total immunoglobulin (Ig) and IgM and the presence of the minor lymphocyte stimulating (Mls) antigens on the surface of these cells. Lymphocytes from the spleens of neonatal and mature immunologically normal and abnormal mice were examined. Using the techniques of rapid flow microfluorometry, they showed that adult mouse splenic B lymphocytes include a large subpopulation of cells with a relatively homogeneous density of total surface Ig. These B lymphocytes, which have a low-to-intermediate density of surface Ig, are not seen in significant numbers in the spleens of neonatal mice. Moreover, their numbers are markedly reduced in the spleens of adult CBA/N mice, a strain with an X-linked defect in B lymphocyte function. They also showed that as B lymphocytes from normal mice mature (in contrast to the CBA/N strain), the density of their surface IgM gradually decreases, so that in the adult mice the majority of these cells have a very low-to-low density of surface IgM.

Previously, they demonstrated that the MLC defined non-H-2 Mls antigens are present on Ig-positive cells from the spleens of adult mice but absent from cells derived from the spleens of neonatal mice. B lymphocytes with a high density of surface Ig from the spleens of adult normal mice also lacked the Mls antigens as did all the cells from the spleens of adult CBA/N mice. These studies defined three surface membrane characteristics that distinguish subpopulations of B lymphocytes which differ in their maturational development.

The fifth and last session of the Conference was concerned with receptors, triggering and maturation of B cells. Howard Grey (National Jewish Hospital, Denver) was the chairman. Studies on simultaneous expression of multiple immunoglobulin classes at different points in B cell differentiation by A. R. Lawton, J. F. Kearney, H. C. Morse III and R. M. Asofsky were given by Dr. Lawton.

The most readily approachable questions on the genetic regulation of B cell differentiation involve the mechanisms through which IgM-synthesizing precursor cells "switch" expression of C_H genes in giving rise to progeny which synthesize other immunoglobulin classes. Without attempting an explanation of this phenomenon, observations in three different systems which relate to this general problem were presented.

LPS, a polyclonal B cell mitogen, is capable of stimulating either newborn or adult mouse lymphocytes to differentiate to mature plasma cells synthesizing IgM, IgG, and IgA immunoglobulins. Using combined techniques of direct immunofluorescent observations and suppression of the response by anti-μ antibodies, they determined that (1) precursors for IgG are probably derived from a small subpopulation of μ-bearing cells which also express surface γ, and (2) restriction is accomplished by loss of cell surface IgM as cells begin to synthesize IgG at a high rate. These observations are consistent with the idea that precursors for IgG synthesis, although they bear μ receptors, are already precommitted to make this switch.

The second system is one in which "switching" appears to be regulated differently. F1 mice immunized with pneumococcal polysaccharide and

simultaneously given parental spleen cells (allogeneic effect) develop substantial IgG and IgA plaque-forming cell (PFC) responses. This response has been analyzed by different techniques for detection of indirect PFC, and by double immunofluorescent staining for all combinations of μ, γ_1, γ_2, and α heavy chains. Their results indicate that a very high proportion of IgM PFC at the peak of the response also secrete IgG_1, while IgM-IgA "doubles" are rare. Immunofluorescent analysis supports these conclusions, and also indicates a surprising frequency of plasma cells synthesizing other combinations ($\gamma_1 + \gamma_2$, γ_1 or $\gamma_2 + \alpha$).

Their final observation involves an induced mouse myeloma, TEPC 609, which after repeated cloning passages produces both IgG_{2b} and IgA immunoglobulins. These two paraproteins have been shown to have the same light chain class and idiotype. By immunofluorescent staining, more than 90% of tumor cells in several generations produce both Ig classes, although "singles" of both classes have always been found as well.

The most important generalization to arise from these observations is that B cells at several distinct stages of differentiation may simultaneously express multiple immunoglobulin classes. This data seems incompatible with models for V-C joining in which a single V gene is sequentially joined to different C genes.

Samuel Strober (Stanford University School of Medicine) next discussed maturation of B cell subpopulations in rats and mice.

Maturation of bursa-equivalent (B) lymphocytes can be divided into two stages: (1) antigen independent and (2) antigen dependent maturation. Virgin and memory B cells are the products of the antigen independent and

antigen dependent developmental sequences respectively. These two sub-populations of B cells differ with respect to migration pattern, tissue distribution, turn-over rate, and class and quantity of cell surface immunoglobulin.

Virgin B cells in the peripheral lymphoid tissue of adult animals are renewed rapidly and continuously by precursor cells in the bone marrow and spleen. Memory cells are renewed slowly, and are found predominantly in the pool of recirculating lymphocytes. Small B cell precursors in the bone marrow are capable of migrating to the spleen, and transforming into large virgin B cells with IgM on the cell surface. Upon contact with antigen the latter cells are capable of giving rise to progeny which secrete IgM but not IgG antibody. However, the more mature virgin B cells (predominantly small cells) give rise to progeny which secrete both classes of antibody. The transformation of small precursor cells in the marrow to large virgin cells in the spleen suggests that there is an antigen-independent clonal expansion of B cells in the peripheral lymphoid tissues which may be involved in the regulation of clone size and renewal.

Joan L. Press spoke on the expression of Ia antigens on hapten specific clonal precursor cells based on experiments done in collaboration with Norman R. Klinman and Hugh O. McDevitt.

These studies were conducted to determine whether the expression of I region associated antigens (Ia) can be used as a marker to delineate B cell subpopulations, and whether Ia antigens play a role in T cell-B cell interaction. Spleen cells from primary and secondary donor BALB.K ($H\text{-}2^{K}$) mice were negatively selected by pretreatment with an A.TH anti-A.TL

(anti-Ia^K) antiserum and complement, then analyzed for precursor cell activity by an *in vitro* splenic focus technique for B cell cloning, which maximizes carrier help for B cell stimulation. The splenic focus technique allows the enumeration of clonal precursor cells in the population with and without negative selection, and permits the analysis of such selection at the level of individual B cells and their clonal progeny. The results of these experiments indicate (a) that almost every secondary precursor cell is Ia positive; (b) that while the majority of primary B cells are Ia positive, a small but finite subpopulation of Ia "negative" primary precursor cells exists in the spleen of non-immune mice; and (c) that the Ia "negative" primary B cells constitute those precursor cells which give rise solely to IgM antibody production, whereas the Ia positive B cell subpopulation contains precursor cells capable of giving rise to IgG, as well as IgM, antibody production. Primary precursor cells can also be stimulated by allogeneic T cells, but then give rise only to IgM antibody production. Since the majority of primary B cells are Ia positive, and have the capacity to give rise to IgG production upon stimulation with syngeneic T cells, it would appear that the Ia positive B cell can give rise either to IgM or to IgG antibody, depending on the stimulatory conditions employed; and that Ia antigens may play a role in the ability of the precursor cell to switch from IgM to IgG antibody production. However, Ia recognitive interactions between B cells and T cells are not obligatory for stimulation to IgM antibody production.

The conference terminated with Marian E. Koshland's discussion of the role of J chain in B lymphocyte differentiation. The studies were done by Veeta Ewan, Elizabeth Mather and herself.

The relationship between J chain synthesis and B cell differentiation was investigated by fractionating rabbit and human lymphoid populations and analyzing their J chain content using radioimmunoassay. When rabbit precursor B cells were separated from normal spleen cells by Sephadex G-10 filtration, an average of 1,300 J chain molecules were found per Ig bearing cell, one-tenth the amount present per B cell in the unfractionated population. This decrease in J chain content was accompanied by a 10-fold decrease in PFC as assayed on TNP-SRBC. A similar depletion was observed with human peripheral blood lymphocytes separated by 1 x g velocity sedimentation. The smallest cell fractions contained 1,000-1,500 molecules of J chain/B cell compared to 1-2 x 10^5/B cell in the large cell fractions. Cytoplasmic fluorescent staining showed that the low levels of J chain present in precursor populations could be accounted for by a few contaminating activated cells (1 in 300). These results indicated that antigen signals *de novo* synthesis of J chain to effect the differentiation of precursor B cells to 19S IgM producers. The repression of J chain synthesis was investigated by examining PFC from rabbits immunized with Lac-BGG. Whereas 90% of direct PFC stained positive for J chain, the percentage of IgG PFC (Con A inhibited, anti-rabbit IgG developed) varied. During active immunization 60-70% of the IgG PFC contained J chain, but after rest and boosting, none were positive. These results suggested that J chain synthesis is turned off in IgG memory cells and perhaps serves as a signal for their dedifferentiation.