Cell Interactions and Receptor Antibodies in Immune Responses

Proceedings of the Third Sigrid Juselius Symposium

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Edited by

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Preface

The third Sigrid Jusélius Symposium was held at the Savings Banks Institute near Helsinki in June, 1970. The proceedings of the symposium are published in this book. Attempting prompt publication we set an early dead-line for manuscripts. Unfortunately this meant omission of some lectures from the book. On the other hand, formal papers were invited covering some points that were central to the topic and were actively discussed at the meeting even though they were not formally presented as a lecture. A great portion of the discussion was omitted from the book. With some exceptions only those contributions are included that were given to the editors written.

The Sigrid Jusélius Foundation is the most important non-governmental supporter of medical research in Finland. It entrusted the scientific responsibility for this Symposium to a group of scientists from the Department of Serology and Bacteriology, Helsinki University. They are identified in the list of participants. This group benefited from discussions with Professor Nils Oker-Blom, scientific secretary to the Foundation who kindly opened the Symposium. Mr Helge von Knorring, until recently Director of the Foundation was responsible for the practical arrangements for the Symposium. His competent assistants were Mrs Barbara Hatcher, Miss Tuula Hindikka and Mrs Hilkka Kontiopää. The work of organizing the symposium was greatly facilitated by the excellent facilities provided by the Savings Banks Institute.

April 1971. Olli Mäkelä, Anne M. Cross, Timo U. Kosunen

Introduction

This is a book about lymphocytes, their classification, characteristics and interactions with other cells. It is greatly concerned with two classes of lymphocytes which were originally distinguished by the work of Szenberg and Warner (*Nature, Lond.* 1962, *194*, 146), Cooper, Peterson and Good (*Nature, Lond.* 1965, *205*, 143), Claman, Chaperon and Triplett (*J. Immunol.* 1966, *97*, 828), Davies *et al.* (*Transplantation* 1967, *5*, 222), Mitchell and Miller (*J. exp. Med.* 1968, *128*, 821) and others. One of the classes was determined by its approximate absence in neonatally thymectomized mice and in normal mouse bone marrow (it may reside in rabbit bone marrow). Another important characteristic of this thymus-dependent lymphocyte is that it cannot transform into a plasma cell. The other class is characterized by its absence from the thymus, presence in neonatally thymectomized mice, relative abundance in the bone marrow and differentiation under the influence of antigen into plasma cells. Both experimental work and observation of human patients (Good and Finstad; Soothill, Kay and Batchelor in this book) suggested that the latter class is responsible for humoral antibody production, while the former class is greatly involved in homograft rejection, and delayed hypersensitivity reactions. An additional function of the latter class is to help in the antigen-induced activation of plasma cell precursors. This introduction will summarize some of the topics that are discussed in the book. Selection of topics was haphazard.

Nomenclature and Characteristics of the Lymphocyte Classes

Table I gives some of the synonyms used for the two lymphocyte classes. The editors decided against making the nomenclature uniform, the names used by the authors are generally maintained. The only exception is that the term "thymus-derived cell" was changed to "thymus-dependent cell". The term "antigen sensitive cell" in this book seems to mean small lymphocytes of both classes.

Both classes of small lymphocytes seem to carry antibody-like receptors for antigens on their surface. In the case of B cells these receptors generally have the same specificity as the antibodies that are produced if the cell is triggered. This is suggested for instance by the papers of Humphrey, Roelants and Willcox;

TABLE I

Nomenclature and characteristics of lymphocyte classes

	Thymus-dependent lymphocytes	Thymus-independent lymphocytes
Other names	Thymus-derived lymphocyte. Antigen-sensitive cell. T cell.	(Bone) marrow derived cell. Antibody-forming cell precursor (AFCP). Plasma cell precursor (PCP). B cell.
Receptors for antigens on cell surface	Demonstrable but probably scarce	Demonstrable
Antigenic markers of classical Ig classes on cell surface	Absent with possible exception of IgM	Demonstrable
Organ-specific antigens theta, Ly^a Ly^b	Present	Absent
Receptors for the third component of complement	Absent?	Present?
Organ-specific antigen PC-1	Absent at least in thymus cells	Present in plasma cells

Mitchison; E. Möller and Greaves; and Wigzell, Andersson, Mäkelä and Walters. B cells may have more receptor antibodies than T cells; this is suggested by the fact that radioactive antigen destroyed plasma cell precursors of the corresponding specificity, but not helper activity (T cells) of the same specificity. This argument is weakened by Miller's finding that irradiated (dead) T cells co-operate *in vitro*. Another finding suggesting scarcity of receptor antibodies in T cells was that Wigzell *et al.* could not deplete helper activity by passing a cell population through antigen columns, although antibody forming activity was arrested.

An important finding suggesting scarcity of antibodies on the T cell surface was made by Raff. Using fluorescent antibodies he could not demonstrate markers of conventional immunoglobulins on cells carrying the T cell marker theta, although they could be demonstrated on theta-negative lymphocytes. On the other hand, using different techniques, Greaves and Hogg as well as Humphrey, Roelants and Willcox produced evidence for IgM and L chain markers on theta-positive cells. Similar findings were made by Ada (*Developmental aspects of Antibody Formation and Structure*, Academic Press, 1970, *2*, 503).

A way to force most of these findings into conformity might be to assume

that T cell receptors belong to a new Ig-class related to IgM. Some anti-IgM sera cross react with it (Ada; Greaves and Hogg; Humphrey, Roelants and Willcox) while others do not (Raff).

There are antigenic markers (Theta, Ly^a, Ly^b) on T cells that cannot be demonstrated on B cells (Raff; E. Möller and Greaves; Greaves and Hogg; Takahashi, Old and Boyse, *J. exp. Med.* 1970, *131*, 1325). Possible markers of B cells are more recent and less firmly documented. They include the complement receptors described by Nussenzweig, Bianco and Dukor. Another possible marker for B cells is the PC-1 antigen, which has not yet been demonstrated in lymphocytes but has been demonstrated in plasma cells (Takahashi, Old and Boyse, *J. exp. Med.*, 1970, *131*, 1325). The third marker reported in September by Raff and Owen (*Proceedings of the 3rd International Congress of Lymphatic Tissue and Germinal Centers in Immune Reactions*) looks very useful. Its antiserum was obtained by immunizing rabbits with bone marrow cells of mice. The donors were thymectomized, lethally irradiated and reconstituted with fetal liver cells.

Preparation of Purified Lymphocyte Classes

Progress is less impressive in preparation of purified T cells or purified B cells. This might be desired for example by students of tumour immunity. A promising possibility is selective complement lysis of T cells from a population using anti-theta, or B cells using anti-Ig or the Raff-Owen antiserum. Nussenzweig, Bianco and Dukor produced lymphocyte populations depleted of complement reactive cells by density gradient centrifugation of rosettes; these may have been B cell depleted populations. Andersson showed that mature T cells can be enriched in the thymus at the expense of immature cells by prior cortisone treatment of the animal. Finally, Miller; Mitchison; and Hartmann prepared "educated thymus cells", which are probably reasonably free of B cells and contain an increased proportion of T cells primed with antigen.

Triggering of Lymphocytes

Lymphocytes can respond to signals in two ways, by proliferating (immune induction), or by committing the equivalent of suicide (tolerance). While the difference between the two signals still remains unknown important data and new ideas were put forward at the meeting. One of them was demonstration by Iverson of a tolerogen which is not immunogenic. It is C3H myeloma protein 5563. This protein injected into syngeneic mice is not immunogenic even in Freund's adjuvant, but coupled with DNP or injected across an allotype barrier, it immunizes mice against an idiotype. Native protein can paralyse cells that are capable of producing the anti-idiotype.

Other important data about triggering were presented by Mitchison. They suggest that T cells are triggered by lower antigen concentrations than B cells (both for immune induction and paralysis induction). This phenomenon could be explained by one of two hypotheses. According to one, the differential affinity hypothesis, T cells have higher affinity receptors than B cells. This has been sponsored by Taylor and Iverson (*Proc. Roy. Soc. Series B,* 1971, *176,* 393). The other, differential triggering hypothesis, is favoured by G. Möller and by Mitchison, mainly because of its power to explain the high number of T lymphocytes reacting to transplantation antigens. The power of their argument is somewhat reduced but not depleted by a finding of Wilson and Nowell, which is supported by findings of Lafferty and Jones (*Austr. J. exp. Biol. Med. Sci.* 1969, *47,* 17). These people found that the high number of reacting T cells is only valid for allogeneic interactions: not many T cells react to xenogeneic transplantation antigens. On the other hand the finding that T cells but not B cells are induced by phytohaemagglutinin (Greaves and Roitt, *Clin. exp. Immunol.* 1968, *3,* 393, Doenhoff, Davies, Leuchars and Wallis, *Proc. Roy. Soc. Lond. B* 1970, *176,* 69) might suggest that the former are more easily triggered than the latter.

Weigle, Chiller and Benjamin demonstrated that both collaborating cell populations, thymus cells and bone marrow cells, exhibit specific tolerance to human gamma globulin. They presented suggestive evidence that tolerance may break down earlier in the B cell population than in T cell population. If the kinetics of paralysis were different in the T and B cell populations this might explain why Taylor (*Transplant. Rev.* 1969, *1,* 114) failed to find specific tolerance in bone marrow cells of paralysed donor mice.

Weigle and his collaborators made rabbits unresponsive to BSA. When they were immunized with serum albumins of other species they produced anti-BSA whose quantity and quality were similar to those of anti-BSA in control rabbits. The authors suggest that the tolerant rabbits lacked only BSA-specific T (helper) cells and not BSA-specific B cells. When human serum albumin, for instance, provided new determinants for the helper function plasma cell precursors against the determinants shared by HSA and BSA were triggered.

Are Post-PCP Clones Restricted to One Ig Class?

While clones deriving from a plasma cell precursor are restricted to one allotype of a locus (Bosma and Weiler, *J. Immunol.* 1970, *104,* 203) and to a maximum of a few antigenic specificities (Wigzell, Andersson, Mäkelä and Walters) they may not be restricted to one class. Most convincing demonstrations that a clone deriving from a plasma cell precursor can make both IgM and IgG are the following: (i) The demonstration by Nossal, Lewis and Warner of single cells synthesizing both IgM and IgG anti-sheep erythrocyte antibodies. (ii) The demonstration of Wang *et al.* (*Proc. Nat. Acad. Sci.* 1970, *66,* 657) that two

para-proteins in one patient, one IgM and the other IgG, had identical (probably H chain) idiotypes and identical N-terminal (27 residues) H chain sequences. (iii) The finding of Oudin and Michel (*J. exp. Med. 1969, 130,* 619) that IgM and IgG antibodies of individual rabbits share idiotypic determinants. It would be attractive to deduce from these findings that the switch from early IgM to late IgG production which occurs at least in anti-erythrocyte responses is caused by a switch within clones and not by selection between clones. The data of Šterzl and Nordin support this deduction. They found a considerable number of spleen colonies which synthesized both IgM and IgG antibody. If it can be demonstrated that the number of B cells and not T cells was limiting in the formation of these colonies this is a very strong argument. Against this deduction is the finding of Cosenza and Nordin (*J. Immunol.* 1970, *104,* 976) that the number of double IgM + IgG cells does not increase at the time of the IgM → IgG switch and is low (0.79% of all antibody producers). Also against this deduction are papers of Wigzell, Andersson, Mäkelä and Walters; Schirrmacher; Mäkelä, Pasanen and Sarvas. They argue that at least a majority of secreted antibody of a given class is produced by cells whose PCP ancestor had receptor antibody of this class, and whose whole progeny mainly produced this Ig class.

Whether specialized IgM and IgG clones are responsible for most immunoglobulin production cannot be decided at present. If the answer is no, a "mobile" gene for the variable region of the H chains might be the cause of doubly active cells or doubly active clones. It might fuse to constant γ and μ chain genes in some cells or float from one the μ gene to the γ gene in another.

Cell Interactions in the Induction of a Humoral Response

An important contribution to the meeting was the bringing together by Raff of two phenomena, the Claman-Miller interaction of two cell types in humoral responses and the Overay-Benacerraf interaction of several different determinants in a humoral anti-hapten response (carrier-specificity). Mitchison, Rajewsky and Taylor (*Developmental Aspects of Antibody Formation and Structure,* ed. J. Šterzl and J. Říha, Academic Press, 1970, *2,* 547) had demonstrated that the basis of the Ovary-Benecerraf phenomenon is a need for hapten-specific cells and carrier-specific helper cells to collaborate in anti-hapten production. They also produced suggestive evidence that the carrier-specific helper cells are the thymus-dependent partners of the Claman-Miller interaction. This was now demonstrated by Raff who showed that the carrier-specific helper cells carry the T cell marker theta.

Collaboration between T cells and B cells was shown to enhance *in vitro* responses to sheep erythrocytes (Hartmann). Both Mitchison and Weigle, Chiller and Benjamin presented evidence that animals with high-zone tolerance lack both B and T cell activities for extended periods.

Several authors produced evidence that this collaboration is not necessary in all humoral responses. Good and Finstad pointed to the high Ig-levels and/or reasonable humoral responses when thymus function is eliminated. Andersson and Blomgren demonstrated fully thymus-independent antigens. Common to them seems to be a small number of different determinants, but a high multiplicity of one kind. Schirrmacher argued that B cells with high affinity receptors are not in need of anti-carrier help and Kontiainen produced evidence that B cells with IgM receptors are less in need of anti-carrier help than B cells with IgG receptors.

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