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CELL COOPERATION IN THE IMMUNE RESPONSE

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INTRODUCTION

The past decade has seen a revolution in cellular immunology, based on the concept of cooperation between different cells in the immune response. Several symposia* and reviews (Lischner & Di George, 1969; Roitt *et al.*, 1969; Richter, 1970; Taylor & Iverson, 1970) have already been devoted to the subject and from the ever increasing number of current papers it can safely be predicted that any attempt to summarize the situation would be largely out of date by the time it appeared. In this article, the aim has been rather to survey the historical background from which the idea of cooperation sprang, to show to what extent the new approach is an improvement on the previous one, and to provide a framework for the understanding of future developments.

I. CELLS INVOLVED IN IMMUNE RESPONSES

1. *The immunologically competent cell*

It has long been recognized that the immune response to foreign substances is of two basic kinds, distinguishable by the reaction to a second contact: immediate hypersensitivity, associated with and transferable by serum antibody, and delayed hypersensitivity (DH), only transferable by, and therefore presumably mediated by, living cells from the lymphoid organs, notably spleen and lymph nodes. The study of skin graft rejection in mice, initiated by Medawar *et al.* (1954) added a third kind of reaction, but since this type of immunity, too, could be easily transferred from immune to normal animals by lymphoid cells and with difficulty, if at all, by serum, it was eventually put in the same category as the delayed response, under the general title of 'cell mediated immunity' (CMI).

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The exploitation by Simonsen (1962) of grafts of spleen and lymph-node cells and of hosts genetically unable to reject them introduced yet another manifestation of CMI, the 'graft versus host' (GVH) reaction, in which changes in the host (splenomegaly, hepatomegaly, wasting) resulted from an unopposed immunological attack by the grafted cells. Since these changes could be accurately quantitated, it was a simple matter to compare the GVH-inducing ability, or 'immunological competence' of different tissues. As in the case of DH, spleen, lymph node, and peripheral blood were the most active, and when Gowans (1962) produced GVH in rats with thoracic duct cells deprived of all but the small lymphocytes, it was the first clear correlation of immunological competence with a single morphological type of cell. Gowans' experiments with thoracic duct drainage (1959) further showed that the immunologically competent cells (ICC) in the spleen and lymph nodes formed a single recirculating population with those in the blood and lymphatic ducts. It is still considered that the ability to initiate CMI lies solely in this recirculating population of small lymphocytes, though the full achievement of the reaction, whether it is to wall off a tuberculous focus or discard a skin graft, may involve 'effector' cells of many other kinds, such as macrophages, polymorphs, and platelets.

Early attempts to localize antibody synthesis, using tissue culture, pointed again to the lymphoid organs. Better culture conditions were soon found to exist in the lethally irradiated animal; 700–1000 rads of X-rays would temporarily suppress the ability of mice, rats, rabbits, and chickens to give a primary response, but this could be 'adoptively' restored by the transfer of genetically suitable spleen or lymph-node cells, which proliferated freely in the depleted organs, particularly the spleen, of the recipient. In rabbits, but much less so in mice, bone marrow cells were also effective (Cochrane & Dixon, 1962). In rats, thoracic duct cells were able to transfer the ability to respond to sheep red blood cells (SRBC), pointing once more to the small lymphocyte (McGregor, McCullagh & Gowans, 1967). Thymus cells were invariably ineffective.

Further clarification followed the introduction of techniques for detecting antibody at the single cell level, notably Jerne's haemolytic plaque method (Jerne & Nordin, 1963), which identified antibody synthesis and secretion in both lymphocytes and plasma cells. In the case of SRBC and other red cell antigens, the first and major site of antibody formation was the spleen, but the route of injection was important, parenteral injection favouring the spleen and subcutaneous injection the draining lymph nodes.

Upon transfer of normal spleen cells to lethally irradiated mice together with SRBC, an almost exclusively splenic plaque-forming cell (PFC) response could be obtained, and by using small enough inocula of donor cells this response could be localized to a few discrete sites in the repopulated spleen, whose number was directly related to the number of donor cells (Playfair, Papermaster & Cole, 1965; Kennedy *et al.*, 1965). These 'haemolytic foci' were interpreted as clones of antibody forming cells, derived from a single precursor, so that a new type of ICC, the 'antibody-forming cell precursor' (AFCP) could be identified functionally, though not morphologically.

The immunocyto-adherence test (Zaalberg, 1964; Biozzi *et al.*, 1964), in which immune spleen cells and SRBC were centrifuged together and formed clusters or 'rosettes', each consisting of one nucleated cell and a halo of SRBC, disclosed a more complex situation. Rosette-forming cells (RFC) were found to be of at least four kinds: lymphocytes (large and small), plasma cells, blast cells, and macrophages (Storb *et al.*, 1969). From studies of the kinetics and specificity of RFC, it was concluded that macrophages form rosettes only by

virtue of passively adsorbed antibody, whilst plasma cells secrete agglutinins in quantity, and lymphocytes and blast cells carry smaller amounts of apparently membrane-bound antibody (Duffus & Allan, 1969). Strong evidence confirming the presence of specific antibody-combining sites on the surface of ICC has come from the use of antigen-coated glass bead columns. Normal or immune mouse spleen cells that passed through the columns were specifically depleted of the ability to adoptively transfer antibody formation against the antigen used on the column, but the missing population of responsive cells could be recovered by elution from the beads (Wigzell & Makela, 1970). A similar depletion can be achieved by rosette formation, followed by density-gradient separation (Brody, 1970).

Other features of the cell-surface antibody of ICC have been detected by antisera against immunoglobulin components. These can be used either to block plaque or rosette formation (McConnell *et al.*, 1969), or to induce blast-cell transformation of lymphocytes (Sell & Gell, 1965), or they can be marked with a fluorescent or radioactive label or coated on to erythrocytes (Wilson, Munro & Coombs, 1969), and allowed to bind to the appropriate cells. By such methods, the presence has been demonstrated on a proportion of splenic and recirculating lymphocytes of immunoglobulin heavy and light chain and allotype determinants (see I.3). Blocking of CMI responses (tuberculin-induced transformation and the GVH reaction) has also been tried; so far only antilight chain sera have been effective (Greaves, Torrigiani & Roitt, 1969; Mason & Warner, 1970). Whether this means that CMI lymphocytes carry only light chains, or buried heavy chains, or heavy chains of a new class, is still in dispute.

2. *The thymus and central lymphoid tissue*

Miller's demonstration (Miller, 1961) that neonatal thymectomy in mice prevented the proper development of all cell-mediated and some antibody responses remains, a decade later, probably the greatest single breakthrough in cellular immunology. Hitherto an entirely mysterious organ, packed with rapidly dividing lymphocytes, whose removal in adult life nevertheless had no apparent consequences, the thymus suddenly stood revealed as a tissue of unique importance, without whose presence in the critical first few days of life an entire subpopulation of lymphocytes failed to materialize, including those in certain defined regions of the spleen (periarteriolar) and lymph nodes (paracortical), the majority of thoracic duct lymphocytes, and about half of those in the peripheral blood (Miller, 1962). The predominant functional deficiency was in the ability to reject grafts, to induce GVH reactions, and to make antibody to some antigens, particularly proteins, bacteria, and erythrocytes. By contrast, little or no impairment was seen in the antibody response to other antigens such as pneumococcal polysaccharides and haemocyanin (Humphrey, Parrott & East, 1964). In the extensively studied case of SRBC, increasing the dose of antigen 100-fold overcame the antibody deficiency (Taylor & Wortis, 1968). More careful study of adult thymectomy showed a slow waning of immunocompetence (Miller, 1965), while if thymectomy was followed by lethal irradiation and bone marrow replacement, immunocompetence was as impaired as in the newborn (Miller, Doak & Cross, 1963). Despite these dramatic effects of thymectomy, the thymus itself appeared to contain few or no ICC, as judged by the induction of GVH or the adoptive transfer of antibody formation, and from this paradox arose the concept of the 'central' lymphoid organ—not itself engaged in immune responses but essential for the correct development of the 'peripheral' organs containing the ICC.

Much debate turned on whether the thymus actually produced the peripheral ICC, or simply matured them in the periphery by means of a humoral secretion. Chromosomally marked cells were found to leave thymus *grafts* and settle in the spleen and lymph nodes, and later, by careful radiolabelling of cells in the intact thymus, a similar peripheralization was shown to occur naturally, especially around the time of birth (Weissman, 1967). However, evidence has also accumulated for a thymic hormone capable of promoting the GVH competence of precursor cells from thymectomized mice (Trainin, Small & Globerson, 1969; Goldstein *et al.*, 1970). It is not yet clear to what extent this humoral activity normally occurs outside the confines of the thymus gland.

Miller's findings nicely complemented those of Good and his colleagues (Good *et al.*, 1962), who had noted that in human congenital immunological deficiency diseases, two systems of cells could be independently affected: a predominantly plasma-cell population—deficient in sex-linked (Bruton's) agammaglobulinaemia—and a population of lymphocytes associated with the thymus—hypoplastic in 'Swiss' agammaglobulinaemia and totally absent in the more recently described Di George syndrome (Di George, 1965). An even more clear-cut distinction between these two functional subpopulations of ICC next emerged in the chicken. It had been known for several years that injection of eggs with testosterone would suppress the development of the Bursa of Fabricius, a cloacal lymphoid organ histologically reminiscent of the mammalian appendix, and also impair antibody responses in the adult bird (Glick, Chang & Japp, 1956). Now it was shown that removal of the bursa or the thymus had quite separate consequences, bursectomy reducing the antibody response and depleting plasma cells and immunoglobulins, but not homograft immunity or DH, and thymectomy having exactly the reverse effect (Warner & Szenberg, 1964). Antibody-forming cells were not found in the bursa, though young bursal cells were shown to be capable of transferring antibody production to irradiated birds (Gilmour, Theis & Thorbeche, 1970). However, just as with the thymus and CMI, the deficit after bursectomy was out of proportion to the modest content of ICC in the organ. Thus the bursa came to be considered as another central lymphoid organ.

It was natural to search for an organ in mammals with the same central function as the bursa, and on the basis of histology, ontogeny, and relationship to the intestine, the appendix and Peyer's patches have attracted the most claims. Removal of some or all of these tissues from rabbits with subsequent irradiation has indeed produced variable antibody deficiencies (Cooper *et al.*, 1966), but it is too soon to say that these mammalian organs should be accepted as true central lymphoid tissue.

'Thymus-dependent' lymphocytes (the ones which fail to develop after neonatal thymectomy) are morphologically similar to other lymphocytes, but it has proved possible to distinguish them by virtue of certain surface properties. The ability to be transformed into blast cells by plant mitogens such as phytohaemagglutinin (PHA) and inactivated by heterologous antithymocyte sera appears to be exclusive to thymus-dependent cells (Doenhoff *et al.*, 1970). In rats a specific allo-antiserum can be raised which recognizes only cells from the thymus itself (Potworowski & Nairn 1967), whilst in mice a system of isoantigens (θ) has been discovered whose expression on lymphocytes seems to be limited to thymus-dependent cells, whether in the thymus or the periphery (Raff & Wortis, 1970). Using fluorescent or cytotoxic assays, the proportion of cells of thymus origin in any organ can be measured; both tests agree that some 40% of spleen cells, 70% of lymph-node or thoracic duct cells, and few or no bone marrow cells carry the θ antigen. Rosette formation by some,

but not all, spleen cells can be blocked by anti-θ antibodies, suggesting that thymus-derived cells can carry antibody receptors and recognize antigen (Schlesinger, 1970).

Another property of lymphocytes that is thought to be associated with the thymus-dependent population is the secretion of soluble factors following interaction with antigen (see III.4). Macrophage-immobilizing, mitogenic, cytotoxic, and many other factors are now recognized (Dumonde *et al.*, 1969).

3. *The bone marrow and haemopoiesis*

Through the use of irradiated mice (see I.1), much has also been learned about the progenitor cells of the haemopoietic and lymphoid systems. At X-ray doses between about 800 and 1000 rads, mice would die within 2 weeks unless protected by an injection of living haemopoietic cells, whose principal usefulness was found to be to restore granulocyte and platelet formation, though erythrocyte and lymphocyte production was also restored; by suitable marker techniques the generation of all these types of cell could be traced to the donor inoculum (Ford *et al.*, 1956). The best restorative cells were found to be bone marrow and, to a lesser extent, spleen and peripheral blood. Lymph node, thymus, and thoracic duct cells were not effective. When small numbers of donor cells were injected, repopulation of the spleen occurred in a focal manner, producing nodules that grew in 10–14 days to a million cells or more—large enough to count with the naked eye (Till & McCulloch, 1961). By a split-radiation method that encouraged the formation of visible chromosome abnormalities in the donor cells, it was shown that each nodule was a single clone (Becker, McCulloch & Till, 1963), deriving from a precursor cell which had many of the attributes of a stem cell and was given the name of 'colony-forming unit' (CFU). Though this was purely a functional description, experiments in which bone marrow was fractionated and the CFU content of the fractions measured, strongly suggested that the CFU was a cell with the appearance of a small lymphocyte (Cudkowicz, Bennett & Shearer, 1964).

The intense study now devoted to these bone marrow-derived spleen colonies led to the following conclusions of relevance here: initially each colony tended to consist of only one type of cell (erythropoietic, granulocytic, or megakaryocytic) but after the 1st week mixed colonies were found; reinjection of the cells from a 'pure' colony into a second host gave rise to colonies of all three kinds; within some colonies, proliferation of new CFU occurred, though these 'second generation' CFU differed from the CFU of marrow in being unable to differentiate except in the spleen, and in having a more limited reproductive potential. There was thus good evidence that bone marrow contained a pluripotential lymphocyte-like stem cell, able to differentiate into the formed elements of the blood, as well as into further stem cells.

The repopulation of the lymphoid organs after irradiation has also been traced to a bone marrow-derived precursor, though discrete nodules have unfortunately not been obtained. When chromosomally marked cells from various sources were injected and their distribution mapped at intervals thereafter, certain definite pathways became apparent. Thymus, spleen, and lymph-node cells would settle in the spleen and lymph nodes, but not in the thymus; by contrast bone marrow cells would colonize spleen, lymph nodes, *and* thymus (Ford & Micklem, 1963) and in extreme cases a single marrow-derived clone of cells has been known to repopulate the entire lymphoid and haemopoietic tissue of the body (Loutit, 1965). In one experiment marked cells from spleen colonies were reinjected into a second host, and found dividing in lymph nodes in response to SRBC (Wu *et al.*, 1968). In another, irradiated mice

were protected with cells from the smallest possible number of spleen colonies (four to thirteen) and challenged a month later with three unrelated antigens. The antibody responses were as good and equal as if much larger numbers of cells had been given, yet all the lymphoid organs contained only donor cells (Trentin *et al.*, 1967)—the inference being that the stem cells were not restricted in the range of their potential antibody repertoire (see III.5).

On the whole, then, the bone marrow may be looked upon as the ultimate source of stem cells in the adult animal, supplying the other haemopoietic and lymphoid organs, via the blood stream, with pluripotential precursors whose further differentiation probably depends chiefly on the prevailing local humoral environment (Wolf & Trentin, 1968). The reduction, but not absence, of lymphocytes in thymus-deprived mice (see I.2) suggests that one such lymphocyte-inducing environment is provided by the thymus, but that other pathways are also available. Following the successful use of anti-θ isoantisera to identify the thymus-derived lymphocytes (see I.2), attempts have been made to raise antisera specific for the non-thymus-derived ones, of which the most promising has been that of Raff *et al.* (1971). Another useful marker for some, but probably not all, of the non-thymus-derived lymphocytes is the presence of immunoglobulin molecules on their surface (see I.1). Using fluorescent or autoradiographic methods (Raff, Sternberg & Taylor, 1970), 40% of spleen cells, and about 20% of lymph-node, bone marrow, and thoracic duct cells were positive (compare with the results for anti-θ in I.2).

4. *Macrophages*

A large part of any injected antigen ends up in macrophages, and years of argument have surrounded the question of whether any of this antigen is made use of in initiating the accompanying immune response. Recently the situation has been somewhat clarified by the demonstration that both antigen-induced transformation and the antibody response to some antigens *in vitro* definitely require the participation of macrophages (Hersh & Harris, 1968, Shorman *et al.*, 1970). Their role in the latter reaction will be considered later (see II.5). Whilst macrophages obviously possess the power of discriminating normal 'self' antigens from their foreign and altered counterparts, there is so far no evidence that this specificity is immunological in the usually accepted sense of an adaptive change in responsiveness, such as would be manifested by memory or tolerence. On the other hand, the participation of macrophages non-specifically drawn into CMI reactions has been clearly shown (Lubaroff & Waksman, 1968). All too little is known at present about the existence of subpopulations of macrophages and the extent of their recirculation.

Throughout this section, repeated emphasis has been laid on the lymphocyte as the basic immunologically competent cell; indeed the development of an immune response, from stem cell to effector, represents a synopsis of the life history of the lymphocyte. During this history, at least two distinct populations of lymphocytes come into being, illustrated most strikingly by the chicken thymus and bursa systems. In the next section it will be shown how these two types of cell can sometimes act not only separately but synergistically.

II. THE EVIDENCE FOR CELL COOPERATION

1. *The carrier effect*

Two curious genetic deficiencies in the immune response first drew attention to the specificity for the carrier in the antibody response to haptens. Guinea-pigs of one strain

could not respond, by antibody or delayed hypersensitivity, to a DNP-polylysine complex, yet if the complex was coupled to BSA, normal responses to the DNP were found (Benacerraf, Green & Paul, 1967). Similarly, some rabbits could make antibody to one of the two subunits of the tetrameric enzyme Lactic Dehydrogenase but not to the other, yet they responded to the hybrid molecule by making antibody against *both* subunits. Thus one subunit could act as a carrier for the other. Animals made tolerant to the carrier subunit no longer responded to the hybrid molecule (Rajewsky *et al.*, 1967). In both systems, simultaneous specific recognition of hapten *and* carrier was clearly essential.

In normal rabbits, a secondary response to a hapten–carrier complex required that the hapten be attached to the same carrier for both primary and secondary challenge. However the hapten could be coupled, the second time, to another carrier provided that the original carrier was also given again, though separately (Rajewsky *et al.*, 1969). The insertion of 'spacer' molecules between hapten and carrier did not impair the immunogenicity of the complex, showing that hapten and carrier were not acting as parts of a single determinant (Mitchison, 1967).

Probing the model further, Mitchison primed mice with various hapten–carrier complexes, or carriers alone, rechallenged their spleen cells *in vitro* with a standard ('right') complex, transferred them to irradiated mice, and tested these for anti-hapten antibodies (Fig. 1). Cells from mice primed to hapten on the 'wrong' carrier were not effective in the second hosts, but responsiveness could be restored by the addition of cells from another mouse primed to the 'right' carrier alone (Mitchison, 1968). This was the clearest evidence for what had been hinted at by the earlier work—that different cells were responding to the carrier and to the hapten, both responses being needed for antihapten antibody to be formed. The fact that the carrier-primed cells are sensitive to anti-θ serum suggests that they are thymus-derived (Raff, 1970).

2. *The premium effect*

A second reason for postulating a two-cell system came from the comparison, in irradiated mice injected with spleen cells and SRBC, of the numbers of haemolytic foci (see I.1) and of PFC. Whereas the number of foci was linearly related to the number of spleen cells injected, the number of PFC increased disproportionately, the slopes of the cell dose–response curves being about 1 and 2 respectively (Gregory & Lajtha, 1968). The simplest explanation would be that the formation of a focus depends on the presence of one type of cell only, whereas the production of a PFC involves the interaction of two cells, neither of which is in overwhelming excess. A similar effect has been noted when culturing peritoneal cells *in vitro* (Bussard & Lurie, 1967).

3. *The failure of thymus-derived cells to make antibody*

As mentioned earlier (see I.2), thymectomized mice irradiated and restored with bone marrow fail to recover the power to respond to many antigens. However, a graft of normal thymus tissue after irradiation allows this recovery. By the use of thymus grafts and bone marrow injections from mice carrying different chromosome markers, or different immunoglobulin allotypes, but otherwise syngeneic, or from not-quite-syngeneic mice, it is possible to 'type' individual cells in the recipients. Employing the latter approach, Davies *et al.* (1967) immunized mice a month after irradiation and grafting, and then transferred their spleen cells to further irradiated mice, some of which were pretreated so as to reject only the

thymus-derived or only the marrow-derived cells. When the thymus-derived cells were rejected the antibody response in the second host was diminished, but when the marrow-derived cells were rejected it was abolished altogether. It was concluded that marrow-derived, but not thymus-derived, cells could make antibody, but that both together could make more. However, despite their failure to make antibody, chromosomally marked thymus-derived

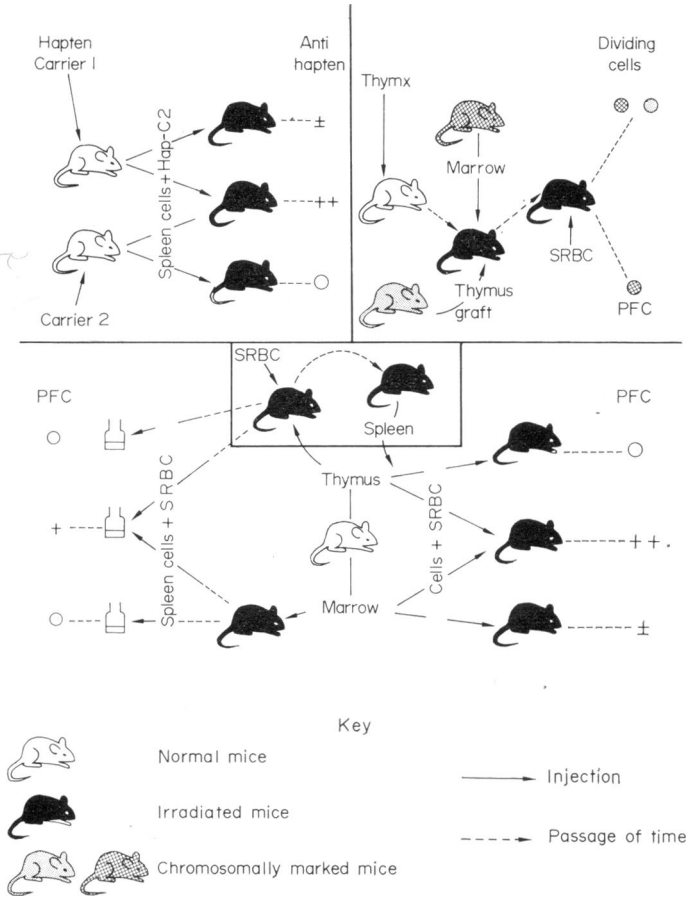

FIG. 1. Experimental models demonstrating cooperation. Top left: The carrier–hapten effect (Mitchison); top right: The failure of thymus-derived cells to make antibody (Davies); bottom: Thymus–marrow cooperation. Left: *in vitro*; right: *in vivo* (Claman); inset: Miller's modification.

cells in the spleen could be shown to divide in response to antigen, mitotic cells of thymus graft origin being significantly increased a few days after an injection of SRBC and many other antigens (Davies *et al.*, 1966) (Fig. 1).

In basically similar experiments, Miller (Mitchell & Miller, 1968) used neonatally thymectomized mice restored with semi-syngeneic thymus cells; isoantisera against those transplantation antigens contained only in the thymus cells did not inhibit the PFC against

SRBC, while antisera against the host antigens did. Facilitation methods, using antisera to bring out PFC making Ig2a antibodies, which can carry donor or host allotypes, also showed the PFC to be of marrow origin (Klein & Herzenberg, 1967).

4. *Thymus-marrow cooperation in vivo*

The simplest and most elegant demonstration of the need for two cell types in the antibody response was an experiment by Claman (Claman, Chaperon & Triplett, 1966), in which cells were injected into irradiated mice together with SRBC (Fig. 1). Thymus cells alone made no foci or PFC, marrow cells made a few, but the mixture gave significantly more of both. Claman (1969) later showed that both the marrow and the thymus cells involved were relatively radiosensitive.

An interesting variation of the above experiment was performed by Miller (1969), who gave the thymus cells to a first irradiated mouse, removed its spleen a week later and injected the cells, together with fresh bone marrow, into a second host. In this 'double transfer' system (Fig. 1) it was found that the antigen (SRBC) had to be given to both hosts in order for the second one to mount an antibody response. Instead of thymus cells, Miller also used thoracic duct cells to cooperate with marrow; in this case, however, the thoracic duct cells alone gave haemolytic foci, the size but not the number of which was increased when marrow was added. As judged by chromosome markers, the PFC were again predominantly of bone marrow origin (Nossal *et al.*, 1968).

With another antigen, polymerized Salmonella flagellin (POL), a rather different result was obtained; bone marrow cells alone were able to form antibody foci, the addition of thoracic duct cells causing no increase in number, though the foci were larger (Armstrong, Diener & Shellam, 1969). It is noteworthy that with this antigen, the tissues richest in focus-forming cells were the Peyer's patches.

Though the unavailability of inbred strains has precluded comparable studies in other species, some evidence for cell cooperation has also been obtained in rabbits. After 800 rads, the antibody response to SRBC was abolished, but could be partly restored by an injection of bone marrow cells. However, the resulting PFC, as judged by antiallotype inhibiting sera, were of *host* type (Richter & Abdou, 1969). When 1000 rads were used, this restoration no longer occurred, and it was postulated that a marrow-derived cell had cooperated with host-derived PFC precursors able to survive 800 but not 1000 rads (Abdou, Rose & Richter, 1969). It may be relevant that with a different antigen, the rabbit appendix has been shown to be richer than the marrow in cells capable of restoring IgM production after irradiation (Hanaoka, Nomoto & Waksman, 1970). In chickens, though the thymus–bursa system might seem ideal for demonstrating cooperation, preliminary results have been unconvincing (Gilmour *et al.*, 1970). These last two examples point to the hazards in generalizing about the source of the various cells involved in cooperation.

5. *Cooperation in vitro*

Recent improvements in tissue-culture techniques have made possible the induction of a primary antibody response *in vitro* (Mishell & Dutton, 1967; Marbrook, 1967). The response by spleen cells is remarkably like that seen *in vivo*, but mixtures of marrow and thymus cells are disappointingly inactive. However, by culturing the thymus and marrow cells separately for a few days in the spleens of irradiated mice (Fig. 1) cell suspensions have been obtained which do demonstrate cooperation when mixed together and cultured *in vitro* (Dutton, 1971).

The tissue-culture approach has also dispersed some of the mystery surrounding the macrophage. Mosier (1967) found that when spleen cells were allowed to settle on plastic, the supernatant cells lost their ability to respond to SRBC *in vitro*. The adherent cells, most of which appeared to be macrophages, also did not respond, but when the two cell populations were recombined, the full normal response was restored. Subsequently it was shown that if the non-adherent cells came from neonatally thymectomized mice there was no response, whereas adherent cells from thymectomized mice behaved normally (Mosier *et al.*, 1970). Addition of thymus cells to the mixture of non-adherent thymectomized cells and adherent cells gave a good response; this constitutes the first example of a three-cell cooperation system—macrophage, thymus-derived cell, and (presumably) marrow-derived cell. The reason why thymus and marrow are sufficient *in vivo* is probably that the host macrophages are still functional, despite irradiation. However, some antigens, such as POL, may not need macrophages at all, even *in vitro* (Shortman *et al.*, 1970).

6. *Cooperation in cell-mediated responses*

It has been claimed that typical CMI responses such as GVH and homograft rejection, also exhibit thymus-marrow cooperation (Cantor & Asofsky, 1970). In many cases it appears that the marrow-derived cell is in fact a macrophage. Cooperation has been demonstrated between macrophages and immune spleen cells in inducing cytotoxicity against tumour cells *in vitro* (Evans & Alexander, 1970). However, the existence of a carrier effect in delayed hypersensitivity (Benacerraf *et al.*, 1967) and the apparent need for more than a single determinant for the induction of delayed skin responses (De Weck & Schneider, 1970) has been cited as evidence that true cooperation among immunocompetent, presumably thymus-derived, cells can occur.

III. MECHANISM AND IMPLICATIONS

1. *Terminology*

In discussing cell cooperation, the term 'immunocompetent cell' is no longer adequate, and it is convenient to have a nomenclature based on the duality of function; many such terms have been coined, of which the following (Roitt *et al.*, 1969) will be used here:

T cell. A thymus or thymus-derived or dependent ICC able to engage in cooperation or CMI, probably a θ-positive cell (see I.2).

B cell. A bone marrow or marrow-derived ICC belonging to the general population of antibody-forming cells and their precursors, and not thymus dependent (see I.3).

ASC. Antigen-sensitive cell: any cell inherently able to recognize a restricted range of antigens.

2. *The function of cells in cooperation*

In the last section, it was shown that in the case of thymus–marrow cooperation in the response to SRBC, the marrow-derived cells ('B cells') actually synthesize the antibody. Moreover, we know that both the immunoglobulin subclass and the allotype of the antibody formed are characteristic of the B cell (Klein & Herzenberg, 1967). Evidently, then, at least the genes for the constant region of the antibody heavy chain are provided by the B cell, which, though not ruling out the possibility of a limited transfer of information from T to B cell, does reduce the likelihood that the T cell alone determines the structure of the antibody

to be made, as once seemed plausible. In any case, the existence of antigens apparently not requiring T cell cooperation argues in favour of the B cell containing all the required information. At present, two hypothetical roles for the T cell are receiving particular consideration:

(a) *Antigen concentration.* According to this view, a critical feature of the stimulation of a B cell is the way the antigen is presented. T cells, by virtue of surface receptors, probably antibody (see I.2), can bind antigen in such a way that the determinants are optimally disposed for finding and stimulating a B cell (Miller, 1969; Taylor, 1969).

(b) *Soluble factors.* As mentioned earlier (see I.2), one of the striking results of antigen recognition by T cells is the release of soluble factors with an effect on other cells. It is an attractive and economical possibility that cooperation with B cells might also be mediated by some such factor.

At present there is no clear proof of either of these two theories. In short, the mechanism of cooperation is still unknown. However, the existence of the problem has stimulated other important questions, some of which will now be discussed in the light of the two above hypotheses.

3. *The site of antigen specificity*

Immunological specificity implies a changed response to the antigen concerned, but not to others. This change may be in the direction of a larger or faster response, which indicates memory, or a smaller or absent response, denoting tolerance. The question is therefore: do T cells or B cells, or both, carry memory and tolerance?

(a) *Memory.* The double transfer experiment of Miller (see II.4), in which thymus cells cultured for a week in the spleen of an irradiated mouse before being mixed with marrow cells, cooperated only if stimulated with SRBC in both hosts, and not if the first host received Horse RBC instead, gave clear evidence for specificity in the T cell population. In a rather similar system, Hartmann (1970) has found that T cells stimulated by Horse RBC will cooperate in the production of SRBC antibodies, but only if Horse RBC are given again with the SRBC. This implies that the stimulation of T cells is indeed specific, but the effect of stimulated T cells upon the B cells may be non-specific (see III.4).

Convincing evidence for memory in the B cell population is scanty; in fact normal immunological memory is conspicuously absent from the response to pneumococcal polysaccharides in mice, where thymus-dependence is minimal and probably absent (Baker & Landy, 1967). It is interesting that in this, presumably a 'pure' B cell response, only IgM antibodies are found (Baker & Stashak, 1969), which, taken with the fact that IgG_1 antibodies are both a prominent feature of the normal secondary response and highly thymus-dependent (Taylor & Wortis, 1968), suggests that immunological memory is at least chiefly a property of the T cell. However, one experiment where primed spleen cells expressed their own IgG2a allotype on transfer, even in the presence of normal bone marrow cells, does raise the possibility that B cells may carry memory (Jacobson, L'Age-Stehr & Herzenberg, 1970).

(b) *Tolerance.* It is an easy matter to take T cells from a specifically tolerant animal and test their ability to cooperate with normal B cells. In all such experiments, this ability has been depressed though the degree of specificity has varied. Thymus grafts from rats tolerant to BGG transferred the tolerance to thymectomized irradiated recipients without affecting the response to ovalbumin (Isakovic, Smith & Waksman, 1965). Thymus cells from mice tolerant to BSA were substantially unable to cooperate with normal marrow cells in irradiated

recipients; however, the response to Human SA was also somewhat diminished. Judged by the ability to restore thymectomized mice, thymus cells became tolerant sooner and more completely than lymph-node cells (Taylor, 1969). A similar experiment with Human-γ-Globulin (HGG) showed thymus cells to be fully tolerant to this antigen but not at all to Burro RBC (Chiller, Habicht & Weigle, 1970). T cells from mice made tolerant by repeated injections of SRBC were found to have a reduced mitotic response to SRBC and also partially to Human RBC (Gershon *et al.*, 1968). In a different system, this 'cross-tolerance' was found between several related RBC, but to an extent apparently out of proportion to the actual degree of cross-reaction (Playfair & Purves, 1971a). Thus there is no doubt that T cells display specificity towards antigens, but this may not always be as great as the specificity of the final antibody response, suggesting that there may be a greater, or a different, source of specificity elsewhere.

Tolerance in the B cell population has been sought in analogous experiments, with conflicting results. In the mice tolerant to BSA (above), and in mice made tolerant to SRBC by the use of cyclophosphamide (Miller & Mitchell, 1970), the marrow cells were able to cooperate with normal thymus in making antibody to the 'tolerizing' antigen. However, when much higher doses of SRBC and of cyclophosphamide were used, the marrow cells were transiently less responsive to SRBC than to Chicken RBC (Playfair, 1969). It has recently been shown that this unresponsiveness lies mainly in the part of the response that is thymus dependent, while the 'direct' response of marrow is hardly reduced. This has been put forward as evidence for two populations of B cells—one responding to SRBC directly (B1) and one only via a T cell (B2) (Playfair & Purves, 1971b). From other experiments it has been further postulated that the B2 cell is only made tolerant in the presence of thymus cells, suggesting that tolerance induction, like antibody induction, involves T-cell cooperation (Gershon & Kondo, 1970). The most clear-cut evidence for B cell tolerance was in the aforementioned mice tolerant to HGG but not Burro RBC. Here the marrow cells were fully tolerant, but more briefly and only when high doses of antigen were used to induce the tolerance (Chiller, Habicht & Weigle, 1971). Pneumococcal polysaccharides can also induce specific tolerance, which presumably affects only B cells, though there is evidence that in this case (and perhaps in all cases) the B cell is not killed but merely prevented from secreting antibody, retaining its surface antibody as shown by the ability to form rosettes (Baker *et al.*, 1971).

Given that only a few antigens and practically no other species than mice have been studied, the consensus seems to be that *both* T and B cells carry specificity, but that the induction of both immunity and tolerance occurs with much greater ease in the T than the B cell. It has been suggested that this relative ease of tolerance induction may in fact be an essential feature of the thymus cell, without which the elimination of potentially autoimmune T cells might not take place (Burnet, 1967). By contrast, the brisk but transient appearance of autoantibodies following tissue damage in normal animals may be a sign that B cells need not always be scrupulously tolerant to 'self' antigens.

4. *The specificity of cooperation*

Even if the response of both B and T cells is antigen specific, the actual cooperative effect could be nonspecific. An example has already been quoted (see III.3) in which activated T cells appeared to stimulate the response of B cells to another antigen. If confirmed, such an effect would inevitably call to mind the action of the soluble factors (see I.2) whose libera-

tion by T cells requires specific antigen but whose effect on macrophages or other lymphocytes is apparently independent of antigen. However, when, in another experiment, mice were primed just before irradiation, giving a subsequently much increased response with injected marrow cells, the priming effect was both thymus-dependent and restricted to the priming antigen (Playfair & Purves, 1971a); if a soluble factor was responsible for the surviving effectiveness of these lethally irradiated T cells, it was an antigen-specific one. In one case the isolation of a soluble 'cooperating factor' has been claimed; cell-free extracts of primed peritoneal cells enhanced the response of normal marrow in irradiated hosts, but only to the priming antigen (Kennedy, Treadwell & Lennox, 1970). No such soluble factor has yet been characterized, but until proved otherwise, antibody seems the most likely candidate. Indeed early IgM antibodies, if uncontaminated by IgG, can enhance the response of intact mice to suboptimal doses of SRBC (Henry & Jerne, 1968), perhaps by passively sensitizing macrophages, which in turn bind antigen in a way favourable to the stimulation of B cells.

An important feature of the normal response to SRBC is the rapid increase of PFC, probably mostly by cell division; in thymectomized mice this increase is less rapid, suggesting that the influence of the T cell might be on the rate of B cell division. Evidence supporting this view has also been obtained *in vitro* (Saunders, 1969). A soluble mitogenic factor would be a neat explanation, but the specificity for antigen will need to be established. It would seem inherently wasteful for every interaction between a T cell and its antigen to be accompanied by division of all the neighbouring B cells, most of which presumably cannot make the appropriate antibody.

5. *The origin of receptor diversity*

The problem of B cell receptor (antibody) diversity will ultimately be solved by the geneticist, armed with the amino acid sequences of all available immunoglobulins. Meanwhile an experiment has already been mentioned (see I.3) which suggested the presence in bone marrow of stem cells multipotent for antibody specificity. To reconcile this with the evidence for B cell monospecificity (III.3), it is necessary to invoke somatic mutation of a small number of inherited genes, or some form of fusion between a large number. The question remains: what is the nature of the T cell receptor, and where is it derived from?

The evidence is incomplete but tantalizing. On the one hand the blocking of CMI reactions by antisera to L-chains, and of RFC of presumed thymus origin by antisera to IgM determinants (see I.2), suggest conventional antibody, perhaps partly buried. On the other hand, it has been shown that the gene controlling the responsiveness of guinea-pigs to an individual carrier (see II.1), almost certainly at the level of the T cell, is closely linked to the genes controlling the major transplantation antigens (Green, 1971), and a similar linkage has been found in mice (McDevitt & Tyan, 1968). This has suggested to some workers that the transplantation antigens on the T cell surface may themselves be the receptors for antigen. This field of study promises an exceptionally interesting future.

The traffic of cells from marrow to thymus is well established (see I.3) while the reverse flow, though evidently possible (Doenhoff *et al.*, 1970), must be relatively small. Thus, if the B cell and T cell receptors are in fact of common origin, the simplest mechanism would be for diversification to occur in the marrow and be carried over into the thymus, particularly since the B cells do not seem to be in any way abnormal in mice deprived of a thymus from birth (Miller & Mitchell, 1968). Yet the massive cell proliferation seen in the thymus has always attracted theorists as an ideal background for a diversification process, especially one

based on random mutation and selection. In the light of the tolerance data (see III.3), an alternative view might be that diversity is generated in the marrow, while cells of undesirable specificity are eliminated in the thymus. A third possibility is that both organs contribute part of the diversification, perhaps by different mechanisms.

6. *Is cooperation useful?*

Since practically all the evidence is derived from cell-transfer experiments, it might be argued that cooperation is an artefact, with no real physiological importance. Certainly it is far from proved that cooperation actually occurs *in vivo* in all the situations where it is theoretically possible. However, the effect of thymectomy on antibody responses (see I.2) and the carrier–hapten effect in intact animals (see II.1) both represent reasonable models for 'real life'; it is quite conceivable that the brisker antibody response to a rapidly dividing bacterium which the intervention of T cells permits, might be life-saving, while the value of the subsequent immunologial memory, largely resident in the T cell population, can hardly be doubted.

A slightly subtler justification has been advanced by Taylor & Iverson (1971), who point out that T–B cell cooperation always seems to require an antigen with two *different* determinants; the need for the same two determinants to be present on the challenging as on the priming antigen would confer great specificity on secondary responses. Conversely, a cross-reacting foreign antigen carrying only one 'self' determinant will be able to initiate a B cell response as long as it also carried a 'non-self' determinant for a T cell to react with. For purely 'self' antigens, there will not normally be any reactive cells available. In this way the margin of safety between unresponsiveness to foreign antigens and excessive autoimmunity is considerably broadened. Following this line of thought, one might predict that tumour cells carrying extra foreign determinants might be disproportionately immunogenic, and in fact there is evidence for an increased immunity, both humoral and cell-mediated, against syngeneic tumour cells either coupled to rabbit γ-globulin (Czajkowski, 1967), incorporating influenza viral antigens (Lindenmann & Klein, 1967), or fused with xenogeneic cells (Watkins & Chen, 1969). It may be premature, but it is still tempting, to see these results as examples of applied cell cooperation.

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