IMMUNOLOGY OF PARASITIC INFECTIONS

Edited by

Sydney Cohen & Elvio Sadun

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Edited by

Sydney Cohen, MD PhD FRCPath
Professor of Chemical Pathology,
Guy's Hospital Medical School, London

and the late

Elvio H. Sadun, PhD
Formerly Head, Department of Medical Zoology,
Walter Reed Army Institute of Research,
Washington, D.C.

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PREFACE

In many tropical and subtropical countries parasitic infections exact a toll of human life and health grave enough to constitute a serious threat to economic and social development. The magnitude of this problem can be illustrated by recent estimates that 300 million people are infected with amoebiasis and 7 million with Chagas' disease; 400 million live in malarial regions with an annual morbidity of 50 million cases and mortality of about 1 million, while the current extension of irrigation projects and growing density of human populations have produced an explosive increase in the incidence of schistosomiasis. In these circumstances the importance of immunoprophylactic measures in parasitic infections need not be stressed. Unfortunately this goal remains elusive and practical success will demand a far deeper understanding of host–parasite interactions than exists at present.

All parasitic infections induce specific antibody synthesis and, as described in Section II, immunodiagnostic tests are of great clinical and epidemiological value. Only rarely, however, does the immune response lead to complete elimination of parasites. As illustrated in Section III, acquired resistance is sometimes not manifest clinically and frequently is associated with persistent, low-grade infection. This state of affairs has been referred to by parasitologists as 'premunition', and represents an equilibrium between host and parasite fundamental for evolutionary survival of parasitic species. Several mechanisms permitting parasite survival in the immunized host have been recognized including antigenic variation and disguise, production of soluble blocking antigens, intracellular location and inhibition of various host defence mechanisms. It remains true, however, that the means whereby parasites evade acquired host immunity are not completely understood for any species. The similarities between continued parasite survival and progressive tumour growths in hosts manifesting potentially lethal immune responses are obvious. Parasitic infections can clearly provide models of great potential value to immunologists interested not only in mechanisms of acquired resistance, but also in the nature of immunopathological complications of disease (Section IV).

These considerations encouraged Dr Elvio Sadun and myself to undertake the assembly of this book in the hope of promoting a greater interchange between parasitologists and immunologists. Towards this end the text includes some relevant basic immunology (Section I) and summaries of the complex life cycles of important human parasites (Appendix). The book was rendered viable by the generous cooperation of many distinguished investigators in the field of parasitic immunology. Dr Sadun himself wrote three invaluable chapters. His tragic death in April 1974 at the age of 55 was a profound shock which left an irreplaceable void for innumerable friends. Whatever merits this book may have are a reflection of Dr Sadun's deep knowledge of the subject, his kindly wisdom and the affectionate esteem which he engendered in colleagues throughout the world.

Sydney Cohen

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LIST OF CONTRIBUTORS

A. C. ALLISON *Division of Cell Pathology, Northwich Park Hospital, Watford Road, Harrow, Middlesex, England.*

R. S. BRAY *Medical Research Council Laboratories, Fajara, Bathhurst, The Gambia.*

K. N. BROWN *National Institute for Medical Research, Mill Hill, London, England.*

S. COHEN *Department of Chemical Pathology, Guy's Hospital, London Bridge, London, England.*

R. R. A. COOMBS *Department of Pathology, University of Birmingham, Birmingham, England.*

D. C. DUMONDE *Division of Immunology, Kennedy Institute of Rheumatology, Hammersmith, London, England.*

R. ELSDON-DEW *State Pathology Laboratory, Edendale Hospital, Private Bag X9020, Pietermaritzburg 3200, South Africa.*

MARC FELDMANN *Imperial Cancer Research Fund Tumour Immunology Unit, Department of Zoology, University College, London, England.*

E. H. FIFE, JR. *Department of serology, Walter Reed Army Institute of Research, Washington, D.C., United States.*

M. A. GEMMELL *Hydatid Research Unit, Medical Research Council of New Zealand, University of Otago Medical School, Dunedin, New Zealand.*

W. L. HANSON *College of Veterinary Medicine, University of Georgia, Athens, Georgia, United States.*

V. HOUBA *Immunology and Research Centre, Department of Pathology, University of Nairobi, Nairobi, Kenya.*

L. JACOBS *National Institute of Health, Bethesda, Maryland, United States.*

R. C. JUNG *Department of Tropical Medicine, Tulane University Medical Centre, 1430 Tulane Avenue, New Orleans, Louisiana, United States.*

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SECTION I

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CHAPTER 1

Cellular Basis of Immune Sensitization

MARC FELDMANN

GENERAL FEATURES OF THE IMMUNE SYSTEM

Two basic responses are considered typical of adaptive immunity, those mediated by humoral antibody, and those mediated directly by the cells of the lymphoid system. Humoral immunity involves the production and secretion of antibody (immunoglobulin molecules capable of reacting avidly with antigen) into the extracellular fluids. Cell-mediated immunity is caused by cells that have become altered (sensitized) as a result of antigenic stimulation. The latter process is responsible for the rejection of most tissue grafts and for delayed hypersensitivity reactions.

The most important cell type in the lymphoid system is the lymphocyte. Lymphocytes are usually round cells with scanty cytoplasm, and are heterogeneous in their properties—size, life-span (days to years), tissue of origin, distribution, and capacity to recirculate through different organs of the body. Lymphocytes can proliferate and differentiate into, for example, blast cells or plasma cells. There are two major categories of lymphocytes, both of which

originate from multipotential stem cells (of marrow, fetal liver, or yolk sac origin) but differentiate by different pathways under the influence of local inducers (Metcalf and Moore, 1971). This differentiation takes place in the 'primary lymphoid organs', the thymus in all vertebrate species, and in birds in the bursa of Fabricius as well. The equivalent of this bursa in other vertebrates has not yet been identified, and it seems unlikely that this function is localized in any definite single anatomical site.

Thymus derived 'T'-cells differentiate in the thymus and from there seed out, via the blood into the T-dependent sites of the lymphoid system (Parrott, de Souza and East, 1966), then through the lymphatics to the thoracic duct and back to the blood (Miller, Mitchell and Weiss, 1967). This line of cells includes thymocytes as 'immature T-cells', peripheral T-cells, and their terminal cell types, 'activated' T-cells which are involved in cell cooperation ('helper cells'), in cell-mediated cytotoxicity ('killer cells') or other functions (e.g. production of lymphokines, etc.).

'Bursal-equivalent derived' cells or 'bone marrow derived' cells are now commonly referred to as B-lymphocytes. The line of cells includes antibody-forming cell precursors, antibody-forming cells, and their terminal cell type, plasma cells. Some of these cells also recirculate, but less than T-cells (Howard, 1972; Sprent, 1973) and through different areas of the lymphoid system (e.g. Mitchell, 1972).

TABLE 1.1. Surface characteristics of T- and B-cells

	B-cells			*T-cells*		
Surface	*Immature B-cell*	*Peripheral B-cell*	*Antibody-forming cell*	*Thymocyte*	*Peripheral T-cell*	*ATC*§
ANTIGEN						
TL	−	−	−	++	−	−
θ	−	−	−	+++	++	+
Ly	−	−	−	++	+	
PC_1	−	−	++	−	−	−
MBLA*	+	++	++	−	−	−
MSLA†	−	?	−	++	+	+
MSPCA‡	±	±	++	−	−	−
H2	++	++	++	+	++	++
RECEPTORS						
Ig	±	+++	+	?	?	?
Fc	−	++	−	±	?	+
C_{3b}	?	++	?	−	−	?

* Mouse-specific B-lymphocyte antigen
† Mouse-specific lymphocyte antigen
‡ Mouse-specific plasma cell antigen
§ Activated T-cell
References to above data may be found in Greaves *et al.* (1973).

T- and B-lymphocytes also differ in many other characteristics. Differences in surface markers between the two cell lineages have been extensively studied because of their practical and experimental importance. These are summarized in Table 1.1, and are reviewed by Raff (1971) and Greaves, Owen and Raff (1973).

LYMPHOID CELL RECEPTORS

Burnet's clonal selection theory (1959) predicts that in an immunocompetent animal there are lymphocytes capable of reacting with almost any exogenous antigen. Jerne (1955) proposed that the only molecule capable of reacting with a high enough degree of specificity with antigen must be antibody. Current concepts incorporate both views, in that antigen-sensitive cells are thought to have antibody-like receptors on their surface. These receptors are of great importance as they account for both *specificity* and *diversity* of immune responses.

Many lines of evidence have accumulated that lymphocytes possess antigen-specific surface receptors. First, labelled antigen binds to a small percentage of lymphocytes from unimmunized animals (Naor and Sulitzeanu, 1967) or form rosettes with red cells. Secondly, lymphoid cell suspensions passed through a column of beads coated with antigen can be selectively depleted of cells capable of responding to that antigen (Wigzell and Andersson, 1969). Immunoglobulin may be found on the surface of B-cells by using labelled (fluorescein or radiolabelled) anti-immunoglobulin (anti-Ig) sera (Raff, Sternberg and Taylor, 1970; Nossal *et al.*, 1972). These sera may inhibit the binding of antigen to cells (Byrt and Ada, 1969) and the induction of B-cell tolerance (Feldmann and Diener, 1971).

Surface labelling studies have permitted the identification of the bulk of receptors on B-cells as 8S IgM molecules (Baur *et al.*, 1971). These studies do not, of course, exclude the possibility that primed B-cells, a minority population, may have IgG receptors (reviewed by Greaves, 1970).

Unlike the situation with B-cells, the chemical nature of the antigen-specific receptor on T-cells is not unequivocally established (see discussion in McDevitt and Landy, 1972). While there is much evidence that T-cells have surface Ig (reviewed by Marchalonis and Cone, 1973) there is also evidence to the contrary (see Crone, Koch and Simonson, 1971; discussions in McDevitt and Landy, 1972 and Vitetta *et al.*, 1972). Alternative candidates for 'the elusive T-cell receptor' consistent with the bulk of the data have been difficult to find, although it has been proposed, without good evidence, that in the mouse Ir (immune response gene), gene products may function as such (discussed in McDevitt and Landy, 1972). Recent evidence indicates that the idiotype (a marker of the combining site) of both T- and B-cell receptors is the same (Wigzell, Binz, Eichmann *et al.*, unpublished data). It remains possible that the receptor on the two cell types, of which the idiotype is a marker, may be linked to two structurally different molecules.

ACCESSORY CELLS

While lymphocytes specifically recognize antigenic stimuli, cells such as macrophages, dendritic reticular cells of lymphoid follicles, monocytes and granulocytes also play important accessory roles in the immune response. Macrophages in particular are of undoubted importance. They have many roles in the production of antibody, such as reduction of antigenic particles to a more suitable size, participation in cell cooperation, controlling the level of extracellular antigen, and may also produce substances which affect the immune responsiveness of lymphocytes. In cell-mediated immunity macrophages are involved in the induction of delayed hypersensitivity (Seeger and Oppenheim, 1972) and also in its expression. Macrophages are important (see Chapter 2) in the killing of bacteria (reviewed by Mackaness, 1971) and in the killing of tumours (e.g. Evans and Alexander, 1972; Matthes and Fischer, 1972).

Dendritic reticular cells of lymphoid follicles retain antigen on the surface of their web-like processes, and it is not phagocytosed. Antigen, in its native form, is thus kept available in the extracellular fluids for long periods of time to any lymphocytes percolating through the area (Nossal *et al.*, 1968). The exact function of this antigen is not known. Speculative functions include immunization for a secondary response (Nossal *et al.*, 1968), cell cooperation between T- and B-cells (Guttman and Weissman, 1972) or low-zone tolerance induction (Ada and Parish, 1968). Some properties of macrophages are summarized in Table 1.2.

TABLE 1.2. Some properties of macrophages

Differentiation sequence	Stem cell–Promonocyte–Monocyte–Macrophage
SURFACE RECEPTORS	
Ig binding	(1) IgG, especially aggregated
	(2) Occasionally IgM
	(3) IgT
C3 binding	Different from lymphocyte C3 receptor
FUNCTIONS	
(*1*) *Humoral*	
Antigen handling	Breakdown to smaller size
	Catabolism
	Presentation on surface
Cooperation	Bind IgT-antigen
	? Release mediators which affect lymphocytes
(*2*) *CMI*	
Induction	(As above)
Expression	Kill bacteria
	Tumours
	Released mediators of CMI
	Involved in granulomas
(*3*) *Miscellaneous*	e.g. Make interferon, complement components

MECHANISM OF IMMUNE SENSITIZATION

Antibody production may proceed by two experimentally separate cellular pathways. Since the progeny of B-cells are the antibody-forming cells, clearly B-cells must always be involved. With some antigens, no other cell types are essential, but with others, two accessory or 'helper' cells are required, namely T-cells and macrophage-like cells (perhaps of the dendritic type).

DIRECT STIMULATION OF B-CELLS

In vitro, many antigens may immunize populations of B-lymphocytes (spleen cells depleted of T-cells and adherent cells). For example, polymeric flagellin, POL, dinitrophenylated POL, DNP POL (reviewed in Feldmann, 1973a), sonicated SRC membranes (Byrd, Feldmann and Palmer, 1974), or antigen-coated beads (Feldmann *et al.*, 1974), lipopolysaccharide LPS (Britton, 1969; Andersson and Blomgren, 1971) may all trigger B-cells directly. *In vivo*, all these antigens, and others, such as pneumococcal polysaccharide Type III, S III (Davies *et al.*, 1971), polyvinyl pyrolidone, PVP (Andersson and Blomgren, 1971) and levan, LE (Miranda, 1972) may elicit responses in the virtual and perhaps complete absence of T-cells. However, it is more difficult to rule out the participation of macrophages in these *in vivo* responses, although by analogy with the *in vitro* results it is difficult to envisage an essential role for these cells *in vivo* which is not crucial *in vitro*.

All these antigens have marked structural resemblances, being large polymers (or surfaces) with repeating antigenic determinants. This particular polymeric structure seems to be of general importance for the stimulation of B-cells, since mitogens which do not stimulate B-cells in their soluble form, namely phytohaemagglutinin (PHA) and Concanavalin A (Con A), may be converted into potent B-cell mitogens by conjugation to a surface. Greaves and Bauminger (1972) conjugated PHA to Sepharose beads, whereas Andersson, Sjöberg and Möller (1972) conjugated Con A to plastic dishes. In an analogous way it was shown by Fanger (1972) that univalent anti-Fab (Fab') antibody fragments did not stimulate rabbit lymphocytes, unlike divalent $F(ab')_2$ fragments. These considerations indicate that a polymeric interaction of antigenic determinants with the surface receptors of B-cells is particularly favourable for stimulation. The exact feature of this interaction which is crucial is not yet beyonddispute. It is apparent that the binding energy of interaction will be much greater, the stability of binding will be greater (Wilson and Feldmann, 1972) and that cross-linking of receptors, with consequent 'patch' and 'cap' formation will occur (Taylor *et al.*, 1971; Diener and Paetku, 1972; Raff, Feldmann and de Petris, 1973). Concurrently the repeating determinants will favour the binding of antigenic determinants to both combining sites of the receptor antibody molecule, a condition which seems to be essential (Feldmann, 1972a), and would favour allosteric conformational

changes in the Fc region of the receptor, as postulated on theoretical grounds by Bretscher and Cohn (1968). These considerations, together with a discussion of other possible mechanisms, have been recently reviewed (Feldmann, 1973a).

CELL COOPERATION

Since the pioneering work of Claman, Miller, Mitchell, Davies and their colleagues in the late 1960s, it is known that interactions between two populations of lymphocytes are of major importance in the generation of many (but not all—see above) antibody responses (reviewed by Claman and Chaperon, 1969; Davies, 1969; Miller, 1972; Feldmann, 1973b). Only in the last two years has it become apparent to what extent the function of these two lymphocyte populations are intertwined. T-lymphocytes regulate the function of B-cells (reviewed by Gershon, 1973), also B-cell products (antibody) regulate T-cell function (reviewed by Uhr and Möller, 1968; Diener and Feldmann, 1972). Macrophages regulate the function of both T- and B-lymphocytes. Responses to certain parasites are thymus dependent (Ogilvie and Jones, 1971; Kelly, 1972).

Observations, both *in vitro* and *in vivo*, have documented that macrophage-like cells and lymphocytes interact. Mosier (1969) noted that clusters of cells usually containing a central macrophage were important in the genesis of antibody production *in vitro*. Matthes, Ax, Fischer and their colleagues (1971) have noted in cinematographic studies of mouse omentum that lymphocytes remain for long periods of time on the surface of dendritic macrophages bearing membrane-bound antigen. Blasts and antibody-forming cells arose in their vicinity. *In vitro* studies have supported the conclusion that cooperation takes place on the surface of macrophages (Feldmann, 1972b).

Cooperation between subpopulations of lymphocytes in antibody production was first noted with sheep erythrocytes (SRC) as antigen (reviewed in Claman and Chaperon, 1969; Miller and Mitchell, 1969). Experiments with hapten-carrier protein conjugates yielded the conclusion that there was cooperation between hapten-reactive cells and carrier-reactive cells (Mitchison, 1971). These were identified as B- and T-cells respectively (Mitchison, 1971).

The mechanism of cell cooperation has been and still is, in part, the subject of controversy. However, the broad outlines now seem clear. The first point to emphasize is that because B-cells make the antibody (Mitchell and Miller, 1968) and also dictate its binding properties, and its biological properties, the other cells must, by definition, act as 'helper cells'. In cell-transfer experiments using hapten-carrier conjugates as antigens it was found that optimally efficient cooperation occurred between primed spleen cell populations only if the hapten and the carrier were linked, i.e. on the same molecule (reviewed by Mitchison, Taylor and Rajewsky, 1970). These experiments provide very strong evidence for a specific component of cell cooperation. Furthermore, they suggest that the immunogen (hapten-carrier conjugate) forms an antigen bridge between T-cell receptors recognizing carrier determinants and B-cells

recognizing haptenic determinants. These experiments do not, however, specify *where* the antigen bridge occurs. One site could be directly between the specific T- and B-lymphocytes, which would have to be in close physical apposition. There are, however, many drawbacks to this mechanism chief amongst which is that T- and B-lymphocytes recirculate through different parts of the lymphoid system (Howard, 1972; Mitchell, 1972; Sprent, 1973) and rare specific cells would be unlikely to meet often enough to amplify ('help') the response. Another site of T–B-receptor linkage may be at the surface of an accessory cell. T-cell receptors could be shed and bound by a third party cell, such as a macrophage or a dendritic cell. Recently much evidence has accumulated (reviewed by Feldmann and Nossal, 1972; Feldmann, 1973b) that the latter alternative is more likely.

Specific mediator of cell collaboration

Feldmann and Basten (1972a) found that mouse T-cells activated (ATC) to a carrier, cooperated efficiently with hapten-primed spleen cells across a cell impermeable nucleopore membrane (pore size 0·2 μm), indicating that co-operation was mediated by subcellular factors. Since the cooperation was specific this factor was also antigen specific. It was found to be a complex of a T-released Ig, termed IgT, with carrier specificity and binding strongly to macrophages (Feldmann, 1972; Feldmann and Basten, 1972a). Subsequent studies identified the specific mediator as similar to 8S IgM (Feldmann, Cone and Marchalonis, 1973). Recently Rieber and Riethmuller (1973) have ingeniously confirmed these findings. Using a rabbit univalent (Fab') anti-mouse Fab preparation as antigen, they obtained supernatants from thymocytes which specifically immunized T-deprived mice to yield a response against rabbit Fab. These complexes were fractionated and the T-cell component was of 7–8S size (and by definition Ig). These complexes were cytophilic for macrophages. Tada and Okamura (1973) have also reported that a rat thymocyte extract may specifically mediate cooperation and contains IgT, which was identified as a monomeric IgM. Gordon (1973) has also found that supernatants from activated T-cells mediate specific cooperation. However, they, like Lachmann and Amos (1971), have yet to characterize the exact nature of their specific mediator.

Non-specific mediators

Hirst and Dutton (1970) and Schimpl and Wecker (1971) have found that allogeneic cells and the supernatants of a mixed lymphocyte reaction (MLR) may augment the response to sheep red cells (SRC). Gorczynski, Miller and Phillips (1972) and Waldmann, Munro and Hunter (1973) found a similar activity in supernatants of activated T-cells stimulated with antigen. Feldmann and Basten (1972b) found that non-specific factors augmented the response to thymus-independent antigens, such as DNP POL to the same degree as to

SRC. In contrast, responses to non-polymeric thymus-dependent antigens, such as DNP fowl γ-globulin (DNP FγG), of T-deprived ('nude') mouse spleen cells, were not augmented in the absence of some specific 'T'-cells. This point has been independently confirmed by Gorczynski, Miller and Phillips (1973) and by Waldmann and Munro (1973a).

Another activity of non-specific T-cell factors in the immune response is to augment the production of IgG antibody. Schimpl and Wecker (1973) found that MLR supernatants augmented the IgG response in 8 or 9 day SRC-primed mice, whose spleen cells were depleted of T-cells by anti-θ and complement and then cultured with SRC. Feldmann (1973c) found that relatively large doses of MLR supernatant increase the IgG response of spleen cells from DNP-primed mice (4–8 weeks previously) provided low doses of antigen were used *in vitro*. Whether these two activities are expressions of the same molecule or of two distinct ones remains to be ascertained. The exact chemical nature of the non-specific factor(s), and their source of origin (T-cell and/or macrophage), are not yet known.

Thus the current concept of cell cooperation involves three cells, T- and B-lymphocytes and an accessory macrophage-like cell (Fig. 1.1), and two types of factors, activated T-cells release IgT, complexed with antigen. This binds to the accessory cell. By analogy with the mechanism of triggering of B-cells with polymeric antigens (see above), it was proposed that IgT-antigen may form lattices on the surface of the third party cell (Fig. 1.1). B-cells would be immunized by reacting with this lattice of antigen, involving linked recognition with T–B-receptors. It should be stressed that this model is fully compatible with current knowledge of lymphocyte traffic and homing patterns (see above), and with the known propensity of B-cells to react with macro-

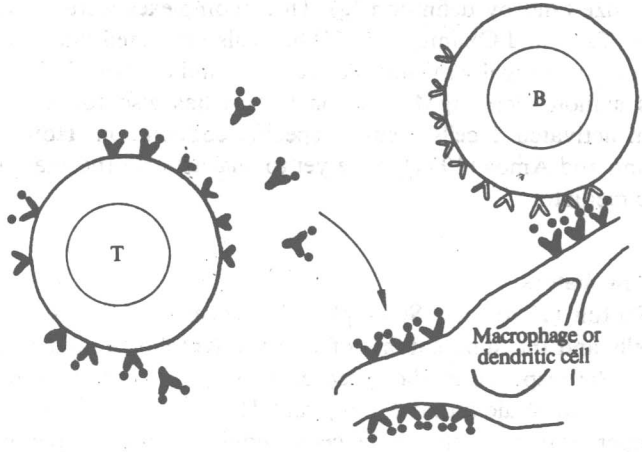

FIG. 1.1. Mechanism of cell cooperation. IgT-antigen complexes are released from the surface of activated T-cells. They bind to the surface of macrophages (or dendritic cells). B-cells are immunized on the surface of these macrophages.

phages (Mosier, 1969; Matthes *et al.*, 1971; Schmidtke and Unanue, 1971, etc.).

Recent experiments on antigenic competition (see below) have verified that lattices of IgT and antigen are formed (Schrader and Feldmann, 1973; Feldmann and Schrader, 1973).

The role of the specific factor would thus be to initiate triggering by a matrix of antigenic determinants. Non-specific factors are of importance to drive the system to proliferate effectively. As Waldman and Munro (1973b) have pointed out, production of non-specific factors by macrophages which have bound IgT rather than by T-cells directly, would facilitate the overall specificity of the system as found *in vivo*.

CELL-MEDIATED IMMUNITY (CMI)

The cellular basis of CMI is even less well understood than that of humoral immunity. Several basic forms have been described. Only those strictly mediated by cells will be discussed. The production of 'lymphokines' which has recently been exhaustively discussed (Lawrence and Landy, 1969, Bloom, 1971) will not be reiterated here (see Chapter 2) nor will the production of the inflammatory lesions of delayed hypersensitivity (see Turk, 1967 and Chapter 27). Three types of CMI will be described differing in the nature of the effector cells.

T-cell-mediated CMI

Allograft functions, both *in vivo* and *in vitro*, are mediated chiefly by T-cells (see *Transplantation Reviews*, Volume 12). The best studied examples are the responses of mice to allogeneic lymphoid cells as measured by the development of 'killer' cells. Brunner, Cerottini and their colleagues (Cerottini and Brunner, 1973) have shown that these responses *in vivo* depend on T-cells, in resemblance to skin graft rejection, which is abnormal in thymectomized mice. *In vitro* studies have confirmed that these responses depend on T-cells (Wagner, Harris and Feldmann, 1972), and that the actual effector cells are T-cells (Goldstein and Blomgren, 1973). These have specificity for the antigen. This form of CMI is specific; bystander cells, even in the same mixture, are not killed. These studies, however, have also indicated that macrophages are also required for T-dependent cytotoxic responses (Wagner *et al.*, 1972). The exact role of macrophages in these responses remains to be determined.

Cooperation between two populations of T-cells has been demonstrated to be important in the genesis of graft-versus-host responses (GVH) in mice (Tigelaar and Asofsky, 1973). Recently experiments have been performed to investigate whether such cooperation also occurred in the generation of T-killer cells. Tigelaar and Feldmann (1973) and Wagner (1973) found that small numbers of lymph node cells dramatically augmented the response of thymocytes to allogeneic cells *in vitro*. Hayry (1973) has shown a similar augmentation of the proliferative response of thymocytes by lymph node cells