Section of Clinical Immunology & Allergy

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Assessment of Immunological Function in Man

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Measurement of Immunological Function in Man: Interaction Between Virus and Human Leukocytes

The importance of cell-mediated immunity in host resistance is well recognized in several virus diseases of man (Allison 1969). The adverse effects of T-lymphocyte deficiency on the course of virus infections is sometimes clinically obvious, as in infants with congenital thymus deficiency who are subjected to vaccination. However, there is growing interest in the possibility that more subtle defects in cell-mediated immunity may be responsible for many chronic diseases of unknown etiology. A ready analogy can be made with chronic bacterial, fungal and parasitic infections which can often be explained on the basis of defects in granulocyte or macrophage function in addition to deficiencies in antibody synthesis. Such defects have usually been detected by *in vitro* techniques measuring cellular reactions with microbial agents. It is predictable that similar *in vitro* methods can be profitably employed to examine the interactions between viruses and mononuclear cells so that the sequence of events by which virus infection is normally limited can be defined and defects of clinical importance can be revealed. Indeed, many of the available techniques have already been applied to this problem.

The range of interactions between viruses and mononuclear cells is depicted in Fig 1. Such schemes are greatly over-simplified because the relative importance of each pathway has to be evaluated in relation to individual viruses. The variable role of each defence mechanism in different virus infections has been well illustrated by experiments involving the adoptive transfer of immunity (Rager-Zisman & Allison 1973). In general, virus antigens are recognized by antigen-sensitive lymphocytes which possess immunologically specific receptors similar to those for non-replicating antigens. Other lymphocyte populations and macrophages limit viral replication by reacting with virus-specific antigens on the surface of infected host cells and with complete virions either in the circulation or during passage from cell to cell. These properties of the effector cells are accentuated by their interaction with antigen-sensitive lymphocytes, particularly after specific immunization. Interactions with serum antibody and complement are also involved.

The *in vitro* assessment of these reactions has now to be considered in more detail. The enumeration of lymphocytes with specific receptors for viral antigens is complicated by the ability of virus to attach to lymphocytes by receptors which differ from conventional antigen-binding sites. Some viruses possess special properties which allow these two kinds of receptor to be distinguished. An example is the hæmagglutinin possessed by myxoviruses and paramyxoviruses which allows lymphocytes with attached virus to be recognized by hæmadsorption with indicator red cells. Using this method, it is possible to characterize two populations of lymphocytes with receptors for influenza virus. The first

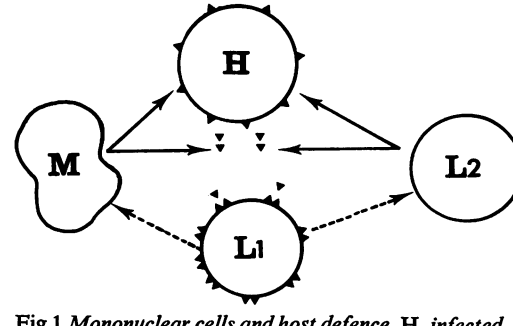

Fig 1 *Mononuclear cells and host defence* H, *infected host cells* ▲, *virus antigen or complete virion.* L 1, *antigen-sensitive lymphocyte with receptors* ▲▲ L 2, *other lymphocytes* M, *macrophage* - - - - → *cooperating pathways.* ——→ *virus-neutralizing pathways*

population comprises 3 to 11% of the lymphoid cells in different human lymphoid tissues and includes cells with a wide spectrum of physical properties. Hæmadsorption by this population is inhibited if the cells are treated with neuraminidase before exposure to virus, suggesting that their receptors are structurally identical with those on other cells and are therefore not peculiar to lymphocytes. The second population, which is expanded as a result of immunization with influenza virus, consists of small lymphocytes with immunoglobulin receptors for virus, and enumeration of these cells gives a true measure of conventional antigen-binding cells. Nevertheless, this assay fails to detect receptors for viral antigens other than hæmagglutinin, which may be of crucial importance in determining the outcome of the infection (Cowan 1973). Another familiar method of assaying antigen-sensitive cells measures the incorporation of tritiated thymidine by lymphocytes stimulated with viral antigens *in vitro* and has been used in patients immunized with influenza vaccine (Denman *et al.* 1970) and with other viruses. Reactivity may at times only be elicited if the cells are challenged with virus-infected cells, cytomegalovirus being a pertinent example (Thurman *et al.* 1973). However, reactivity may not be revealed by blast transformation once the peak of infection has been passed. Mention must also be made of migration inhibition (Utermohlen & Zabriskie 1973) – a technique which is simple in concept, but trying in practice.

There is *in vitro* evidence that some viruses are directly inactivated by lymphocytes. The ability of many viruses to replicate in lymphocytes stimulated by phytohæmagglutinin or other mitogens has been frequently described (Wheelock & Toy 1973). The majority of reports have emphasized the results of experiments in which virus titres, after an initial eclipse phase, increased significantly above those of the original inoculum. Little attention has been given to any other outcome of the interaction between lymphocytes and viruses, although the failure of 17D yellow-fever virus to replicate in the phytohæmagglutinin-stimulated lymphocytes of subjects specifically immunized against this agent has been described (Wheelock *et al.* 1971). It is clear that replication is not the only outcome of infecting blood mononuclear cell cultures with different viruses (Denman *et al.* 1974). PHA-stimulated cultures from normal subjects were exposed to a variety of viruses. Virus titres were assayed daily and, as a control, compared with those of the same virus cultured in the presence of cells killed by repeated freezing and thawing. These experiments showed that some viruses replicated under these conditions, while others lost infectivity dictated by thermal inactivation alone, since the decline in virus titre was identical in the presence of killed or viable cells. A third group was inactivated very rapidly after exposure to stimulated lymphocytes and, to a lesser extent, by unstimulated cells. The data from these experiments are summarized in Table 1. The inactivation of influenza virus by lymphocytes has been studied extensively (Zisman & Denman 1973) and it seems likely from these and ultrastructural studies (Hackemann *et al.* 1974) that virus enters the cell, is uncoated within cytoplasmic vesicles, and thereafter undergoes an incomplete cycle of replication. These lymphocytes appear to be a different population from the antigen-sensitive lymphocytes. This process has distinct advantages for the host since it not only leads to localization and inactivation of virus replicated by permissive cells, which, for influenza virus, are the lining epithelial cells of the respiratory tract, but also, perhaps, to efficient processing of antigens within lymphoid cells. The prompt inactivation of influenza virus by lymphocytes even from subjects who lack detectable anti-viral antibody calls in question the immunological specificity of the reaction. However, it is likely that some residual sensitization to such a commonly encountered agent invariably persists, though at times it may be detectable only by carefully performed blast transformation studies with purified influenza antigens (Cole 1974). The antigen-sensitive lymphocytes cooperate with other lymphocytes to increase the efficiency of virus inactivation. The regularity with which stimulated cultures from normal subjects support the replication of certain viruses nevertheless obscures a more complicated picture because of the heterogeneity of cell populations within the cultures. Herpes simplex virus replicates in predictable fashion, but the overall increase in titre obscures the fact that not all lymphocytes show this permissiveness. In separation experiments a lymphocyte population can be isolated which inactivates this virus despite the increased titres obtained in cultures of unseparated cells. Furthermore, this population expands following clinical infection with herpes simplex virus, so that blood mononuclear cells are no longer capable of supporting the replication of herpes simplex virus irrespective of the mitogen used to stimulate them. Cultures from such patients

Table 1

Fate of viruses exposed to human blood mononuclear cells

Replicated	*Inactivated*	*No effect*
Herpes simplex	Influenza	Adenovirus
Vaccinia	Sendai	Rhinovirus
Echo	Coxsackie B3	Reovirus
Polio	Measles	
Vesicular stomatitis		

Several strains were tested. Fate of virus in PHA-stimulated cultures from normal human subjects is compared with that of virus exposed to frozen and thawed cells

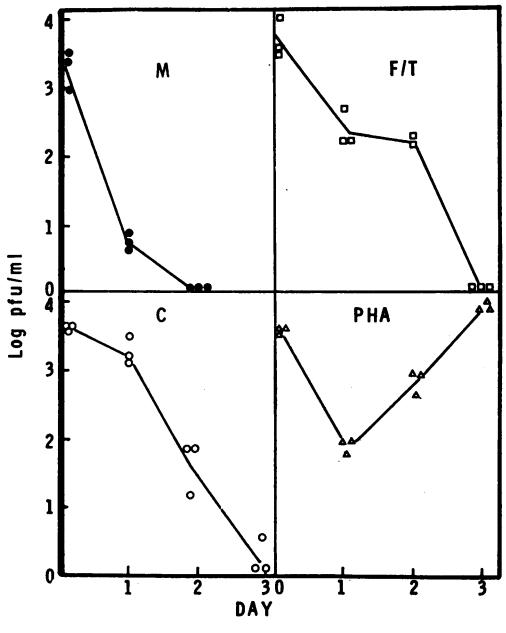

Fig 2 *Reproducibility of assay for herpes simplex virus replication. Infectivity measured on chick embryo fibroblasts in triplicate on each day.* M, *medium alone.* F/T, *frozen and thawed cells.* C, *control, unstimulated lymphocytes.* PHA, *phytohæmagglutinin-stimulated lymphocytes.* pfu, *plaque-forming units*

retain their permissiveness for other viruses, indicating that their altered behaviour towards herpes simplex virus is determined by immunological factors. This specificity makes it unlikely that interferon accounts for the acquired resistance to herpes simplex infection – a conclusion supported by measurement of interferon secreted into these cultures which lags considerably behind the inactivation of herpes simplex virus. The altered behaviour of lymphocyte cultures towards virus replication can be readily applied to the search for a viral etiology in diseases where an infectious agent is not immediately apparent. Moreover, the sensitivity of semi-micro plaque assays (Rager-Zisman & Merigan 1973), allied to their reproducibility (Fig 2), have simplified the clinical use of such techniques.

Considerable attention has been given to the possibility that cell-mediated immunity operates through cytotoxic reactions against virus-infected cells (Porter 1971). To this end, several assays have been devised for measuring the ability of blood lymphocytes and other mononuclear cells to release ^{51}Cr from virus-infected target cells (Steele *et al.* 1973). In principle, combinations of virus strain and host cells are employed which allow the expression of virus-specific antigens on the cell membrane, but which produce only a slow cytopathic effect. Particularly noteworthy are cytotoxic reactions by sensitized lymphocytes against cells infected with measles or rubella virus. Such techniques help in the interpretation of a number of puzzling phenomena in human immunopathology. For example, the atypical cells in the blood of patients with infectious mononucleosis show spontaneous and efficient cytotoxicity against lymphoblastoid cell lines with surface antigens coded by EB virus (Denman & Pelton 1974). Nevertheless, firm evidence is lacking that cell-mediated immunity operates *in vivo* against infected cells rather than directly to inactivate infectious virions in the blood or other extracellular compartments.

The interactions between virus and lymphoid cells are complicated by the variable ability of these cells to support virus replication. Furthermore, the *in vivo* relevance of many of these reactions remains unproven. Nevertheless, it is already clear that *in vitro* techniques are available for assessing many of the pathways and cooperative mechanisms which constitute cell-mediated immunity to virus infections in man.

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Assessment of Antibody-producing Capacity in Man [*Abstract*]

ΦX 174 is a highly immunogenic bacteriophage which can be used to measure the capacity for antibody-production in man. The results of intravenous immunization in 26 normal adults have already been described (Peacock *et al.* 1973).