THE IMMUNE RESPONSE TO VIRAL INFECTIONS

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Edited by

B. A. Askonas
National Institute for Medical Research
London, United Kingdom

B. Moss
National Institutes of Health
Bethesda, Maryland

G. Torrigiani
World Health Organization
Geneva, Switzerland

and

S. Gorini
Fondazione Internazionale Menarini
Florence, Italy

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PREFACE

Virus diseases continue to represent serious health problems in most parts of the world. In spite of the fact that diseases such as poliomyelitis and measles have been controlled in the industrialized countries by vaccination, vaccines now in use in tropical countries have proved not to be optimal. Further research is needed to develop new vaccines that will be effective in all countries. To do so we need to understand better the immune response to different viruses so that we may be able to maximize the protective response of new vaccines and minimize their potential immunopathologic effect.

An exciting new discovery which is now being further developed is the possibility of being able to use some viruses (e.g. vaccinia, adenoviruses, etc.), as carriers for other antigens. This may open up the way for the production of vaccines that will be inexpensive and that will confer long-lasting immunity after only one injection.

This meeting has also served to review our present knowledge of virus diseases which are still of great importance such as hepatitis, dengue and influenza.

G. Torrigiani

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THE CONTRIBUTIONS OF VIRUS INFECTIONS TO OUR UNDERSTANDING OF THE IMMUNE SYSTEM

G.L. Ada

World Health Organization
Geneva, Switzerland

Viruses, on the one hand, symbolise mankind's greatest achievements in controlling infectious diseases yet on the other hand, they still constitute a very great public health risk and provide a scientific challenge to make effective, safe and inexpensive vaccines. Vaccines against smallpox, yellow fever, polyomyelitis, measles and rubella have been or are very successful. Smallpox has been eradicated and transmission of measles reduced to extremely low levels in the USA following the need for parents to provide evidence of vaccination prior to admittance of their children to school. Immunisation with current vaccines to control measles and poliomyelitis is sufficiently effective to suggest that in principle measles could be eradicated and there is a plan to eradicate poliomyelitis in the Americas. In contrast, there are other viral infections of global importance for which either current vaccines are inadequate or too costly or vaccines are unavailable. These include the following viruses - herpes, cytomegalo, dengue, hepatitis A, B and non-A non-B, rota, influenza, parainfluenza, respiratory syncytial and retro viruses, particularly HTLV III. Viral infections of the G.I. and respiratory tracts are major killers of young children, particularly in third world countries. There is still concern that an influenza re-assortant virus might arise which could cause a pandemic Dengue and the hepatitis viruses are major pathogens in tropical countries, particularly in South-East Asia. Herpes and cytomegaloviruses are continuing problems in all populations especially in immunocompromised people. The HTL viruses now are a major challenge as they may attack cells of the immune system and show great antigenic variation.

There is also increasing evidence that viruses may be the causative agents in a number of common diseases such as cancers eg. Epstein-Barr virus in Burkitt's lymphoma, Hepatitis B virus in primary hepatoma, papilloma virus in cervical cancer and HTLV I in leukaemia. Other viruses have been implicated in diabetes mellitus (Coxsackie virus), multiple sclerosis (HTLV I) and rheumatoid arthritis (E.B. virus).

Viruses may be grouped according to their disease pattern. Thus, some viruses cause acute infections i.e. there is no evidence of virus persistence or post-recovery trauma in non-compromised hosts; examples are influenza and pox viruses. Some viruses may cause chronic and/or persisting infections in apparently normal individuals; examples

are measles, cytomegalo and adeno viruses. Other viruses may cause latent infections which may, as seen above, result in neoplasms; examples are herpes, hepatitis B, retro and lenti viruses. The pathology observed during and after primary infection may therefore differ widely.

On the other side of the coin, some viruses have been remarkably effective as tools for unravelling the pathways of cellular metabolism and replication e.g. adenovirus. Others, because of the potential hazard of causing pandemics have been very thoroughly studied by a variety of techniques e.g. the haemagglutinin of the influenza virus is one of the most studied of all proteins. Viruses have also been very important tools for understanding how the immune system works - in fact, as the latter most likely evolved to combat infectious agents of which viruses are an important group, it is not surprising that an important arm of the immune response - effector T cells with cytotoxic activity - evolved mainly if not completely to control many viral infections by aiding in the recovery process. The first demonstration of this phenomenon soon led to a fundamental discovery in Immunology - how the T lymphocyte receptor recognises foreing antigens.

Recently, the "practical" and "academic" aspects of the study of viruses their pattern of infection and the subsequent immune response have come together in a remarkable fashion. The ability to manipulate DNA to form recombinant molecules is having and will continue to have a profound effect on the future directions of scientific research. It is now possible to make recombinant viruses which upon infection of a cell, result in the expression of the inserted "foreign" DNA. Viruses so far used for this purpose include vaccinia, herpes and adeno viruses. As an example, DNA coding for specific antigens of about 10 different infectious, including viruses and parasites, have been incorporated into vaccinia virus. When used to immunise hosts in model systems, some recombinant preparations have protected the host animal from death when challenged with many lethal doses of the agent from which the "inserted" DNA was derived. There is thus a bright prospect for the use of such recombinant viruses as the basis of future vaccines against a variety of infectious agents.

Such recombinant viruses are also proving to be a powerful tool for seeking answers to other questions eg. what antigens of a virus are preferentially recognized by T lymphocytes? They may be vectors of DNA coding for a variety of other substances, such as cellular antigens, growth factors and hormones and so on. Transfection of cells with DNA may achieve the same purpose but recombinant viruses may prove to be a more effective means of achieving desired effects in vivo.

The study of viruses is demonstrably important from several aspects. This Symposium will feature presentations which illustrate many of the more important aspects mentioned in this statment.

CD4 AS THE RECEPTOR FOR RETROVIRUSES OF THE HTLV FAMILY: IMMUNOPATHOGENETIC IMPLICATIONS

M. Carbonari, M. Fiorilli, I. Mezzaroma,
M. Cherchi, and F. Aiuti

Department of Allergy and Clinical Immunology
University of Rome "La Sapienza"
Rome, Italy

INTRODUCTION

CD4 is the HIV (Human immunodeficiency virus type I) receptor, and the virus seems to bind to it through the Leu3a/OKT4a epitope (Klatzmann et al., 1985). HIV infection may lead to the destruction of CD4+ cells both by direct and indirect mechanisms. Direct mechanisms include the formation of syncitia among infected cells (Lifson et al., 1986). Indirect mechanisms may involve autoimmune reactions, of both cell mediated and humoral types (Klatzmann and Montagnier, 1986).

A number of autoantibodies are produced by HIV-infected individuals; they include anti-phospholipid, anti-platelet and antilymphocyte antibodies. Some of such autoantibodies seem to induce significant pathological abnormalities, such as thrombocytopenia (Stricker et al., 1985) and, possibly, lymphopenia (Ho et al., 1987). However, the pathogenetic role of antilymphocyte antibodies has been only poorly defined.

Another intriguing point concerning the role of CD4 in the pathogenesis of HIV-related dysorders, is that cells other than T lymphocytes (e.g. macrophages, cells of the central nervous system and EBV-infected B-cells) appear to express this molecule. These non-T CD4+ cells might be both target for HIV-induced destruction and reservoir for the virus.

To further investigate the problems addressed in the above paragraphs, we performed the studies that we are reporting here.

RESULTS AND DISCUSSION

Antilymphocyte antibodies in HIV infection

Little is known about the mechanisms which lead to the production of autoantibodies in HIV infection. Polyclonal B-cell activation by HIV antigens is likely to be involved (Pahwa et al., 1985), but other mechanisms might also be of importance. Autoantibodies to CD4+ cells, described by some authors (Dorsett et al., 1985), might be evoked as a part of an antidiotypic circuitry to the CD4-binding region of the viral envelope glycoprotein (Del Guercio and Zanetti, 1987). However, this type of autoantibodies (e.g. to CD4) have been, so far, only very poorly

characterized. Therefore, we examined HIV+ sera to evaluate the presence ad clinical significance of antilymphocyte antibodies.

Contrary to previous reports (Dorsett et al., 1985; Tomar et al., 1985), we found no evidence for antibodies reactive with large populations of normal T-cells in any of the sera examined. However, we found that most of the sera had antibodies staining a small (3-5%) subset of peripheral blood lymphocytes from normal donors. Technical details and the representative results from one experiment are described in Fig. 1. A preliminary characterization of the cells stained by HIV+ sera suggested them as being CD3, CD4, CD8-negative, and HLA-DR positive. However, technical considerations (the need for strong compensation of red fluorescence, to avoid leaking of green fluorescence) precluded to us to firmly rule out a (weak) CD3 positivity. Thus, it is likely that we were detecting the autoantibody to the 18 Kd T-cell activation antigen described by Stricker et al., 1987. A major discrepancy between our data and previous reports (Dorsett et al., 1985; Tomar et al., 1985), is that we were unable to identify autoantibodies reacting with large populations of T-cells. This probably depends on technical differences: in fact, we put particular care in reducing, by electronic compensation, the leaking of the strong green fluorescence of polyclonal anti-human Ig antibodies. In fact, a critical re-evaluation of a published report (Tomar et al. 1985) of antilymphocyte antibodies, detected by FACS analysis, suggests that the data may have been biased by insufficient compensation of fluorescence analysis.

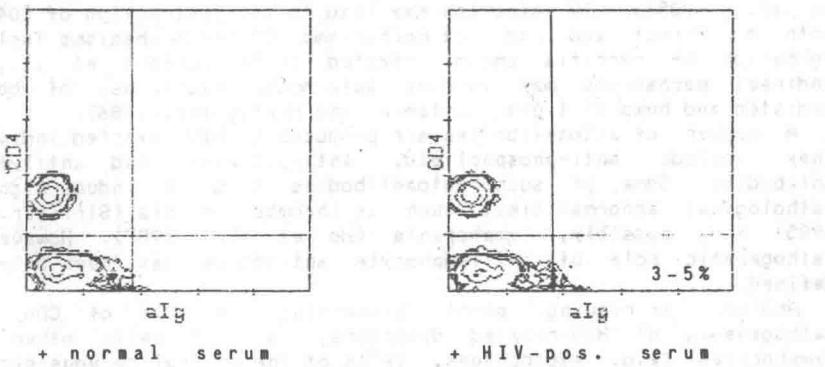

Fig. 1. HIV-seropositive (right panel) or seronegative (left panel) sera were incubated with normal human peripheral blood lymphocytes; cells were counterstained with Leu3a (red fluorescence) or anti-human IgG antiserum (green fluorescence). Anti-IgG alone was used to set the vertical gating (not shown). In this representative experiment, 3.4% of lymphocytes were stained by HIV+ serum but not by the normal serum. Consistent results were obtained with eight HIV+ (from seropositive asymptomatic, AIDS or AIDS-related complex patients) and five HIV- sera.

Our data, while questioning the existence of antibodies to CD4 or to other major T-cell populations, are compatible with the presence of autoantibodies to a discrete subpopulation of activated T-cells in HIV-infected individuals (Stricker et al., 1987). How these autoantibodies are induced by HIV infection is a matter for speculation. Besides polyclonal B-cell activation (Pahwa et al., 1985), a model for this autoimmune response might be the following. HIV-infected individuals are known as having large numbers of activated T-cells in

vivo (i.e., T-cells expressing class II MHC products) (Fig. 2). We examined the distribution of HLA-DR positive cells among CD4+ and CD8+ T-cell subpopulations, and found grossly the same distribution among these two subsets (Fig. 2).

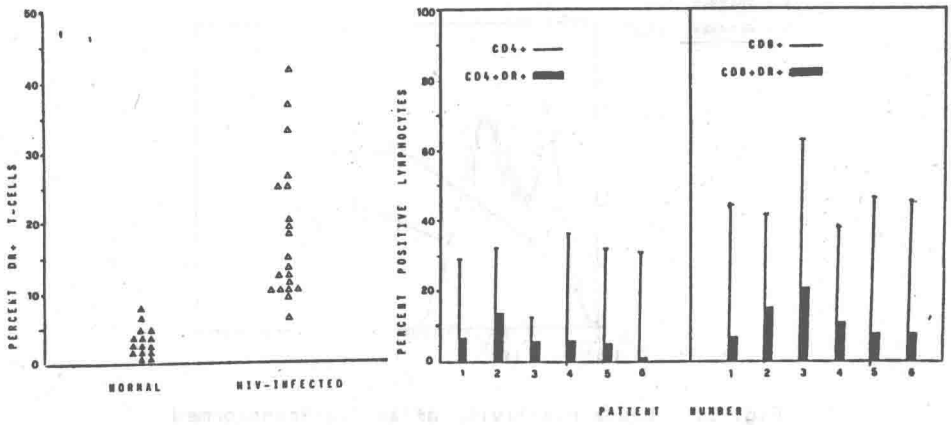

Fig. 2. Percentage of HLA-DR positive T-cells (CD3+) in normal and HIV-infected subjects (left panel), and their distribution between CD4+ and CD8+ subsets (left panel).

Thus activated, MHC class II-positive, CD4-positive cells are abundant in HIV+ individuals. These cells might be also coated with HIV antigens (either produced endogenously by an infected cell, or absorbed as soluble antigen from body fluids by uninfected cells). Viral antigen(s) present on the cell surface might, in turn, bind some membrane structure(s) (e.g. the 18 Kd molecule expressed by T cells during activation) and render it (auto)immunogenic by steric modification. This modified autoantigen would then be efficiently self-presented, owing to te presence of class II MHC products on the cell surface.

Expression of CD4 outside T lymphocytes

A point of major significance concerning the role of CD4 in the expression of HIV infection, is its presence on cells other than T lymphocytes. So far, monocyte/macrophages, some cells of the central nervous system, and some EBV-transformed B-cells have been found to express CD4. While macrophages appear to bear the same CD4 molecule of T-lymphocytes (Stewart et al., 1986), cells of the nervous system (glial cells and some neurones) express a truncated form of CD4 mRNA, which is perhaps not even translated into protein (Maddon, 1986; Gorman et al., 1987). The CD4 expressed by some EBV-transformed B-cell lines (Salahuddin, 1987) has not been, so far, characterized. As the role of CD4 in terms of infectability of EBV-B cell lines by HIV is unclear (Salahuddin, 1987), we undertook a molecular characterization of the CD4 molecule expressed by an EBV-transformed lymphoblastoid cell line obtained in our laboratory from the B cells of a normal donor. This cell line (named blym-2) has 50-80% CD4-positive cells (Fig. 3). Immunoprecipitation studies and FACS analysis showed that it bears a typical 55 Kd rMW CD4 species, carrying all of the following epitopes: Leu3a, OKT4, OKT4a, OKT4b, OKT4d, OKT4e. Thus, some EBV-infected B cells bear the same CD4 as T cells, including the putative attachment site for

HIV (i.e. the Leu3a epitope). These cells may therefore represent, in vivo, a potentially important reservoir for HIV, expecially considering their proliferative capacity. This might account for the recently recognized role for EBV coinfection on the expression of HIV-related pathologic manifestations (Ragona et al., 1986).

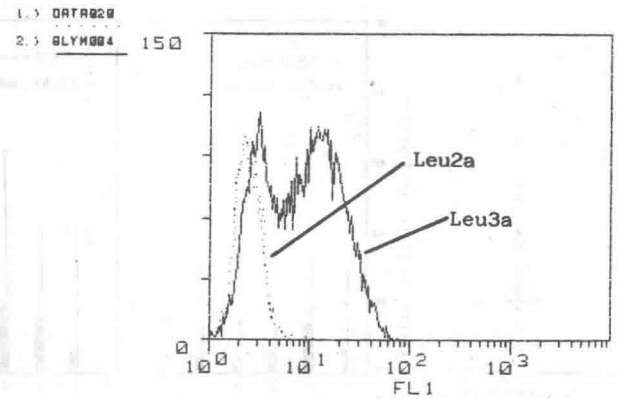

Fig. 3. Leu3a positivity of an EBV-transformed B cell line (blym-2).

It is unclear why only some EBV-transformed B-cell lines (about 15%) express CD4. On one hand, normal circulating B cells do not appear to express detectable CD4 (our unpublished data). On the other hand, EBV superinfection does not seem to induce CD4 on CD4-negative B-cell lines (Salahuddin, 1987). Understanding of the relationship between EBV infection, induction of CD4 on transformed B-cells, and expression of HIV infection, might highlight a significant co-factor for the development of AIDS.

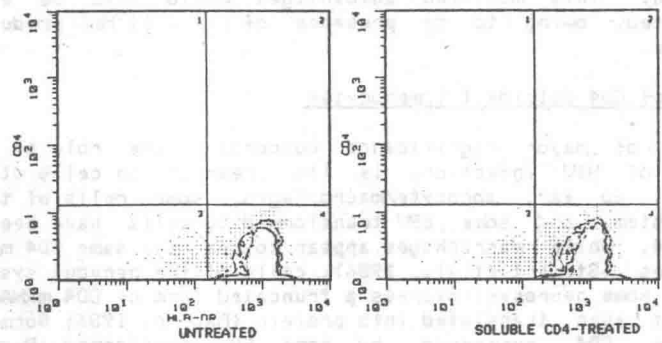

Fig. 4. Soluble CD4 does not bind surface class II MHC products. Cells from an EBV-transformed B cell line (HLA-DR positive, CD4 negative) were incubated at one million/ml with soluble CD4 (kindly provided by E. Reinherz) at 2 ug/ml final concentration (or with saline as control), for one hour in the cold. Then, cells were washed and stained with FITC-labelled anti-CD4 (Leu3a+Leu3b, Multiclone, Becton-Dickinson) and with anti-HLA-DR monoclonal antibodies.

In addition, since CD4 in soluble form has recently been demonstrated capable of inhibiting viral binding to CD4+ lymphocytes and, thus, of neutralizing HIV-1 infectivity, its use in vivo as a therapeutical agent has been hypothesized (Weiss, 1988). However, a potential side effect might be its interference with the functioning of the immune system via its interaction with MHC class-II molecules. We, therefore, performed experiments to assess whether CD4 might bind to MHC class-II products expressed by a (CD4-negative) EBV-transformed B cell line.

Preliminary results (Fig. 4) suggest that soluble CD4 does not bind HLA-DR; thus, it probably would not interfere with normal antigen presentation. Whether this might occur in vitro using other test systems, or upon in vivo administration of soluble CD4, has still to be defined.

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T-CELLS IN RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION

B.A. Askonas, P. Openshaw and M. Cannon

National Institute for Medical Research
The Ridgeway, Mill Hill
London NW7 1AA

INTRODUCTION

T-cells are an important arm of our anti-viral defense, and generally are required to clear virus once a virulent infection has occurred. In certain virus infections T-cells can cause immunopathology. For development of more rational vaccines it becomes important to acquire knowledge about the role of T-cell subpopulations in infection, their viral recognition as well as the induction requirements of protective T-cell responses.

RSV is a paramyxovirus belonging to the genus Pneumovirus. RSV infection occurs regularly in winter and causes serious disease in infants and also in old people. It is the most common single cause of admission to hospital of otherwise normal infants in their first year of life (for example review by Stott & Taylor[1]). Most RSV deaths are in children with cardiac abnormalities and these children are more susceptible to the most severe bronchiolitis. In view of its seriousness for infants, RSV has been designated for vaccine development by WHO and there is a special WHO program for respiratory paramyxovirus infections of childhood. Maternal antibodies appear to protect against RSV only for the first 6-8 weeks and this contrasts far longer term protection against many other viral infections; although high levels of certain types of neutralising antibodies can be protective[2], overall there is no correlation between antibody titres and protective immunity. Moreover, early trials with formaldehyde inactivated vaccines (which induced neutralising serum antibodies) failed to protect and resulted in exacerbated disease and high morbidity when vaccinated children were exposed to natural infection[3].

Adults get reinfected with RSV every few years although neutralising serum antibodies are present.

There are indications that T-cells are important in recovery from RSV infection in as far as T-cell deficient individuals suffer from severe RSV infection, and the virus infection persists in athymic nude mice[4]. Isaacs et al.[5] find that infants with severe bronchiolitis show very low anti RSV CTL activity in culture from PBL. On the other hand the strong lymphoid cell infiltration observed in the lung in bronchiolitis may well contribute to the immuno-pathology.

These problems prompted us to study T-cell mediated immune responses, their role in RSV infection as well as the viral recognition patterns by cytotoxic T-cells (CTL) and T-helper cells (T_H). Although it is possible to study recognition of viral proteins by T-cells in vitro in both mouse and man, we still require animal models to examine the function of T-cell subsets in vivo in infection. Knowledge in this respect is essential to permit development of safe and protective vaccines against RSV. Since the age group of children most susceptible to bronchiolitis is 8-24 weeks, it is unlikely that vaccination using any attenuated live virus preparation will fill the bill. We therefore need an animal model in order to define protective immune responses, to test the immunogenic potential of vaccines, and the immune recognition of the virus and its components. We have used a mouse model for our studies; mice support RSV replication in the lungs after intranasal infection with some human isolates of RSV (e.g. the A2 strain), with histological changes not dissimilar to human pulmonary infection[6]. The infected mice show little or no overt illness, and lethal RSV infection in normal mice has not been demonstrated even with high doses of infectious virus. After RSV infection of BALB/c mice, virus titres in the lung peak days 4-5; imunocompetent mice clear the virus by day 10[6], while in athymic or irradiated mice a persistent infection is established.

RSV specific CTL are detected in lung by day 5 of infection[7], at an earlier time than virus specific antibodies which appear from day 9 onwards. By 2 weeks of infection memory T_H and CTL cells can be recovered from the spleen. Memory CTL in spleen can be stimulated in vitro with RSV infected syngeneic spleen cells as antigen presenting cells and after 5 days' culture T-cell mediated cytotoxicity is generated and the ability to lyse MHC compatible RSV infected target cells (tumour cells and