



Jean-Paul COUTELIER, Associate Member

Laurent DETALLE, Graduate Student

Dan SU, Graduate Student

Nadia OULED HADDOU, Technician

Claude MAUYEN (part time), Technician

VIRAL IMMUNITY AND PATHOGENESIS

The possibility for evolved organisms to survive viral infections depends on the ability of their immune system to eliminate the infectious agent. Therefore, numerous mechanisms, involving different types of immune cells such as cytolytic lymphocytes, T helper and B lymphocytes and macrophages, the molecules that allow those cells to communicate, namely the lymphokines, and the products of those interactions, including antibodies, have been elaborated. On the other hand, viruses have developed strategies to escape the immune system of their hosts, such as large frequencies of mutations or latency, or even to impair this system, which often leads to diseases such as autoimmunity or immunodeficiencies. Our project is to analyse, in murine models, some aspects of these relations between viruses and the immune system.

VIRAL INFECTIONS RESULT IN A DRAMATIC INCREASE IN THE PROPORTION OF IGG2A

Of particular interest is the fact that all antibody responses are not equal. Indeed, depending on their isotype, immunoglobulins display various properties. For example, IgG1, one of the major IgG subclass in mice, cannot fix the complement, contrary to IgG2a, another major component of murine immunoglobulins. Such a difference may lead to dramatic variations in the functional effect of antibodies, as their ability to lyse cells they have bound. During the last few years, we found that the isotype of antibody responses was influenced by concomitant viral infections. The effect of the virus resulted in a dramatic increase in the proportion of IgG2a, not only in antiviral antibodies,

but also in immunoglobulins with an antigenic target unrelated to viral proteins. A dual regulation of antibody responses by gamma-interferon (IFN- γ) and interleukin-6 explains this isotypic bias (1). In the case of antiviral antibodies, a possible explanation for this phenomenon could be the selection by the infected host of the most appropriate response against the virus. Using a model of infection with lactate dehydrogenase-elevating virus (LDV) (2), we could demonstrate that IgG2a antiviral antibodies are indeed more efficient than other isotypes to protect mice against a fatal polioencephalomyelitis induced by the virus (3). The advantage for the host to select IgG2a in non-antiviral responses is more difficult to understand. In addition, the modification of the isotype of antibodies reacting with self antigens could potentially lead to more deleterious autoimmune reactions. This property of viruses to enhance selectively the production

of one immunoglobulin isotype could depend on the preferential activation of a subset of T helper lymphocytes. Indeed, different subpopulations of those cells, called Th1 and Th2, respectively, are distinguished in particular by their capability of producing selectively IFN- γ or interleukin-4, which can selectively trigger B lymphocytes to produce IgG2a or IgG1, respectively.

ACTIVATION OF NATURAL KILLER CELLS

Many of the influences that viruses may have on diverse immune responses can be explained by the production of pro-inflammatory cytokines, including IFN- γ . Therefore, our analysis of the relationship between viruses and the immune system has focused on the activation, by LDV, of cells from the innate immune system that are able to secrete this cytokine, namely the natural killer (NK) cells. Within a few days after infection, a strong and transient NK cell activation, characterized by accumulation of this cell population in the spleen, by enhanced IFN- γ message expression and production, as well as by cytolysis of target cell lines was observed (4). Two pathways of IFN- γ production have been observed that both involve NK cells. The first pathway, found in normal mice, is independent from type I IFN and from interleukin-12. The second pathway involves interleukin-12, but is suppressed by type I IFN. Because NK cells and IFN- γ may participate in the defense against viral infection, we analyzed their possible role in the control of LDV titers, with a new agglutination assay. Our results indicate that neither the cytolytic activity of NK cells nor the IFN- γ secretion affect the early and rapid viral replication that follows LDV inoculation.

Similarly, infection with mouse hepatitis virus (MHV) is followed by NK cell activation and leads to IFN- γ production by those acti-

vated cells. In contrast to LDV, MHV replication is controlled by this cytokine and animals unresponsive to this molecule quickly die after infection. The protective effect of IFN- γ appears to target infected cells rather than lymphocytes.

Interestingly, NK cell activation results in an increased expression of CD66a (CEACAM-1), an adhesion molecule that serves also as a receptor for MHV. However, this enhanced expression, that is also found on immature NK cells, results from NK cell stimulation with IL-12 and IL-18, but not with LDV (5). Therefore, some, but not all subpopulations of activated NK cells might be susceptible to MHV infection.

ACTIVATION OF MACROPHAGES AND AUTOIMMUNE DISEASES

Activation of cells of the innate immune system includes also macrophages and leads to an enhanced phagocytic activity, with potential detrimental consequences for ongoing autoimmune diseases. Our analysis has been focused on autoantibody-mediated blood autoimmune diseases. A new experimental model of anti-platelet response was developed in the mouse (6). Immunization of CBA/Ht mice with rat platelets was followed by a transient thrombocytopenia and production of autoantibodies that react with epitope(s) shared by rat and mouse platelets. In contrast, BALB/C mice similarly immunized with rat platelets did not develop thrombocytopenia. We have analysed whether a viral infection could modulate such an autoantibody-mediated autoimmune disease. In mice treated with anti-platelet antibodies at a dose insufficient to induce clinical disease by themselves, infection with LDV or mouse hepatitis virus was followed by severe thrombocytopenia (7). Similarly, administration of anti-erythrocyte monoclonal autoantibody to mice resulted in the development of

a transient hemolytic anemia that was dramatically enhanced by a simultaneous infection with LDV, leading to the death of most animals. This viral infection induced an increase in the ability of macrophages to phagocytose *in vitro* autoantibody-coated red cells, and an enhancement of erythrophagocytosis in the liver (8). Treatment of thrombopenic or anemic mice with clodronate-containing liposomes and with total IgG indicated that opsonized platelets and erythrocytes were cleared by macrophages. Administration of clodronate-containing liposomes decreased also the *in vitro* phagocytosis of autoantibody-coated red cells by macrophages from LDV-infected animals. The increase of thrombocytopenia triggered by LDV after administration of anti-platelet antibodies was largely suppressed in animals deficient for IFN- γ receptor. Together, these results suggest that viruses may exacerbate autoantibody-mediated thrombocytopenia and anemia by activating macrophages through IFN- γ production, a mechanism that may account for the pathogenic similarities of multiple infectious agents. Regulation of macrophage activation results in modulation of autoantibody-mediated cell destruction and may be considered as a possible treatment for autoimmune diseases that involve phagocytosis as a pathogenic mechanism. Together, these two models may correspond the development of some auto-immune diseases : a first stimulus triggers the production of autoantibodies, through molecular mimicry. A second stimulus, such as a viral infection, leads to the activation of macrophages and results in the destruction of opsonized target cells (9).

Macrophage activation by LDV led also to an enhanced response to lipopolysaccharide (LPS), and to an exacerbate susceptibility to endotoxin shock (10). A synergistic effect of LDV and LPS triggered dramatic production of tumor necrosis factor (TNF) and IFN- γ . Susceptibility to LPS shock was completely mediated by TNF, and partially by IFN- γ .

Therefore, our model of infection with LDV illustrates how a virus can modulate an ongoing disease initially independent from the infection through modification of the immune microenvironment of its host. In this context, the production of type I IFNs may protect the host against exacerbated pathology by controlling the production of IFN- γ .

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Jean-Paul Coutelier

de Duve Institute
Av. Hippocrate 74-75
B - 1200 Brussels

[T] +32 02 764 74 37

[F] +32 02 764 74 30

[E] jean-paul.coutelier@uclouvain.be

[W] http://www.deduveinstitute.be/viral_immunity.php