Lymphology 23 (1990) 56-59

IMMUNOSUPPRESSIVE ACTIONS OF RETROVIRUSES

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ABSTRACT

The immunosuppressive properties of retroviruses were first demonstrated by Old et al. We later showed that Gross Passage A retrovirus superinfection in mice resulted in decreased antibody production and diminished allograft rejection. We have studied in some detail the immunosuppression which occurs subsequent to infection with feline leukemia virus (FeLV) as characterized by profoundly depressed T and B lymphocyte responses and decreased production of γ-interferon. Injection of staphylococcal protein A (SPA) corrected these deficient immune responses, cleared circulating FeLV from blood and produced a regression of FeLV-induced lymphomas and leukemias. The immunosuppressive properties of FeLV and certain other retroviruses have been linked to the transmembrane viral envelope peptide, p15E. Cianciolo et al synthesized a 17-amino acid viral component which shares sequence homology with a highly conserved region of p15E. In vitro *analyses have shown that this synthetic retroviral peptide suppresses T and B cell functions, inhibits the generation of cytotoxic lymphocyte (CTL) responses and dramatically alters the morphology and distribution of monocytes. The latter finding, along with reports that cells of the monocyte/macrophage lineage play a critical role in the initiation of human immunodeficiency infection, suggests that monocytes and macrophages may play a crucial role in retroviral infection and some of the associated immunodeficiencies associated with retroviral infection.*

The immunosuppressive effects of animal and human retroviruses have been known since the late 1950s, when Old and Clark first reported in a Federation Proceedings abstract and a New York Academy of Sciences paper that the Friend leukemia virus could inhibit formation of hemagglutinating antibodies against foreign red blood cells (1,2). From studies in our laboratory in Minnesota during the early 1960's, Peterson, Hendrickson and Good (3), Dent, Peterson and Good (4), and Dent (5) reported that infection with the Gross Passage A retrovirus inhibited antibody production and depressed allograft rejection in C3H mice. Numerous *in vitro* and *in vivo* suppressive actions have been established for the Gross, Friend, Rauscher and Moloney retroviruses (6,7) as well as for the feline and murine leukemia viruses (8-10). Recent attention to the pathology and progression of human retrovirus infection, especially with the lethal human immunodeficiency virus, has elucidated many more of the complex interactions of viruses and viral proteins with immune system components and function.

Our interest in retroviruses and their immunosuppressive properties has been further fueled by our analyses and those of others studying feline leukemia virus in cats. Olson et al (8), Orosz et al (9,10) and our group (11,12) have reported that inactivated FeLV inhibits T-cell proliferation, plaque-forming cell responses and

interferon production *in vitro*. We found that infection with UV-irradiated FeLV caused a significant decrease in γ-interferon production and resulted in regression of FeLV-induced lymphomas or leukemias (15), which was accompanied first by elevated γ-interferon levels in circulating blood followed by cytotoxic complement dependent antibody responses to lymphocytes infected with feline leukemia virus that were bearing gp70 antigen at their surfaces. Rejection of the leukemia cells often but not always was accompanied by elimination of circulating virus.

In collaboration with Savita and Rajendra Pahwa and Gallo's group at the National Cancer Institute, we investigated an essential paradox of HIV infection: namely, that while as few as 1:10,000-1,000,000 cells are at first productively infected with HIV, lymphoid cell populations and immune system functions at the same time may be profoundly depressed by HIV infection. Further, even relatively early in such infections evidence for polyclonal B cell activation was reflected in high circulating levels of some or all immunoglobulins. We studied the influence of band-purified, inactivated virus, and retroviral components and reported that both can dramatically stimulate polyclonal B cell activation and at the same time inhibit both B and T cell functions *in vitro* (16). We also found that polyclonal B cell proliferation, which could be inhibited by stronger concentrations of the viral preparations, is T-cell dependent and to some degree is independent of monocyte regulation (17). Since noninfectious virus preparations resulted in depression of immunological functions *in vitro* that mimicked the immunodeficiency, which accompanied actual HIV infection, we concluded that viral proteins may be responsible for some of the immune dysregulation that occurs early in HIV infection. A significant role of the envelope protein gp120 in the pathogenesis of immune suppression by HIV added further credence to this theory (18).

One of the most thoroughly analyzed of the viral peptides that appears likely to be responsible for the immunosuppressive actions of retroviruses is the transmembrane envelope protein p15E. This transmembrane component of relatively low molecular weight is present on many transforming type C retroviruses, including the feline and murine leukemia viruses and the HTLV-I and HTLV-II viruses that cause T cell leukemias in humans. Cianciolo et al (19) as well as others (20-21) have found that a hydrophilic 26 amino acid region of p15E from numerous transforming retroviruses shares amino acid sequence homologies of 73 percent or greater (HIV also has been shown to share some homology, about 35 percent, in a comparable region of this virus). Cianciolo, Synderman and colleagues (22) then synthesized a 17 amino acid peptide (CKS-17) that is homologous with the highly conserved region of p15E. When coupled to bovine serum albumin as a carrier protein, this molecule has been found in our studies to be impressively immunosuppressive. In analyses employing this synthetic peptide, we observed with Mitani that this peptide, like the irradiated FeLV, strongly suppressed polyclonal B cell activation without compromising viability of the lymphocytes (23). It is also of interest that cells of cancer cell lines, but not normal lymphoid cells, may be immunoreactive with epitopes of p15E (24).

With Ogasawara, we have shown that the synthetic CK-17 inhibits the *in vitro* synthesis of γIFN by peripheral blood lymphocytes, even when exposure to CKS-17 is terminated before final culture (25). More recently, we found that this suppression could be abrogated to some degree by treatment of the cells with IL-1 or IL-2, but especially by combined stimulation with IL-1 and IL-2 (Ogasawara, M, GJ Cianciolo, R Snyderman, M Mitani, NK Kizaki, RA Good, NK Day: manuscript submitted).

We have also observed that CKS-17 inhibits the generation of cytotoxic lymphocyte (CTL) responses, possibly as a result of the inability of helper T cells to produce helper factors required for proliferation and differentiation of CTLs. This

inhibition of CTLs to alloantigens may be significant in the pathogenesis of retroviral-induced immunosuppression, since CTLs are believed to play an important role in allograft rejection, tumor surveillance, and resistance to viral infections (Ogasawara, M, GJ Cianciolo, R Snyderman, M Mitani, RA Good, NK Day: manuscript submitted). We also noted that IL-2 and, to a lesser extent, IL-4 abrogated the suppression of CTL responses, suggesting a role for cytokines in modulation of immune response by retroviruses. In other studies of the biological role of CKS-17 and its interaction with IL-2, we have found that CKS-17 inhibits production of IL-2 by peripheral blood lymphocytes and expression of IL-2 receptors on peripheral blood lymphocytes that have been stimulated with staphylococcal enterotoxin A (Ogasawara, M GJ Cianciolo, R Snyderman, M Mitani, T Kizaki, RA Good, NK Day: unpublished report). These observations suggest that p15E, particularly the homologous hydrophilic amino acid sequence present in p15E that is represented by CKS-17, may be of importance in inducing at least in part immunosuppressive actions attributed to the transforming retroviruses.

Finally, we have noted with great interest that even brief incubation with CKS-17 results in dramatic changes in the morphology and distribution of monocytes (Liu, WT, RA Good, S Haraguchi, GJ Cianciolo, NK Day: unpublished report). We and other investigators have thus begun to regard monocytes as a major target of retroviral infection. Salahuddin and colleagues (26) showed that the monocyte/macrophage cells could be infected by HIV. However, unlike T lymphocytes, monocyte/macrophage function does not appear to be impaired by the binding of and infection with HIV. Koenig et al (27) have reported that circulating infected monocyte/macrophage cells may play a critical role in the initiation of HIV infection in the brain and HIV-related neuropathy. As Fauci suggests, cells of the monocyte/macrophage lineage might be a principal mode of infection of T4 lymphocytes with HIV (28). Our observation that the crucial amino acid sequence contained in p15E can markedly affect cells of the monocyte/macrophage lineage which in turn induce immunosuppression strengthens the theory that monocytes may be an important target of retroviral infection and that injury or stimulation of monocytes may generate components which suppress immunologic responses of T lymphocytes.

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