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Retroviral Immunology: Lessons from a mouse model

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Abstract

Friend virus is a murine retrovirus that causes acute disease leading to lethal erythroleukemia in most strains of mice. Strains of mice that mount the proper immune responses can recover from acute infection, but develop life-long chronic infections. The study of this infection has revealed the types of immune responses required for both recovery from the acute phase and the control of the chronic phase of infection. This knowledge has led to vaccines and therapeutics to prevent and treat infections and associated disease states. The FV model has provided insights into immunological mechanisms found to be relevant to human infections with viruses such as HIV-1 and HTLV-1.

Keywords

Regulatory T cells; suppression; retrovirus; immunotherapy; CD8+ T cells; chronic viral infection; Friend virus

Overview

The Retroviral Immunology Section of the Laboratory of Persistent Viral Diseases uses a mouse model of retrovirus infection to study basic mechanisms of immunological resistance. The primary virus studied is Friend virus (FV), a retroviral complex discovered in leukemic mice by Charlotte Friend in 1957 [1]. FV is an oncogenic virus classified as a gammaretrovirus. It induces erythroleukemia in most strains of mice, but strains vary considerably in their relative susceptibility and resistance. Comparative studies of these strains has led to the discovery of the immunological responses that mediate recovery from acute FV infection, that control chronic FV infections, and that are required for vaccine-induced protection. Our studies have also led to investigations of virus escape mechanisms during chronic infection and the development of immunotherapies designed to reactivate immunological responses for the clearance of persistent viruses.

Acute Infection

Acute infection by FV is controlled by both immunological and non-immunological mechanisms [2]Hasenkrug, 1997 #6}. Our lab has focused on the immunological aspects of resistance during acute infection to determine which lymphocyte subsets and immunological mediators are required for recovery. Early experiments with T cell subset-depleted mice demonstrated that both CD4+ and CD8+ T cells were critical for recovery from acute infection [3]. We also showed that virus-specific antibodies were essential for recovery and could induce recovery even after establishment of infection[4]. However, the efficacy of

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antibodies was dependent on intact CD4⁺ and CD8⁺ T cell responses. The requirement for both B cell and T cell responses held true even in mice with a high level of genetic resistance to FV infection [5]. Studies of numerous cytokines showed predominant roles for IL-12 and IFN γ in recovery from acute infection [6] and mice deficient in IFN γ failed to control FV infections long term. [7]. The main theme that has emerged from these studies is that a successful immunological response to acute FV infection requires the engagement of multiple arms of both the innate and adaptive immune systems.

Resistance Genes

The study of retroviral resistance genes provides important insights into areas of virus weakness, which the host can take advantage of. The role of host genes in counteracting retroviral pathogens has been studied extensively in the FV model and numerous genes have been described that contribute either to initial control of or recovery from FV infection (reviewed in [2,8]). Many of these genes are major histocompatibility complex (MHC) genes, but several non-MHC genes were also found to influence recovery and immune responses. In 1979 Bruce Chesebro described a single autosomal, non-MHC gene that controlled the ability of mice to recover from FV viremia and prevent leukemia [9]. This gene was named **Recovery from FV, gene 3** (Rfv3) and was shown to control viremia and the development of virus-neutralizing antibody responses [10]. As the development of retrovirus-neutralizing antibodies is of extreme interest to the HIV field, we have been very interested in determining the identity of the Rfv3 gene. Genetic studies were used to map Rfv3 to a 0.83 cM region of chromosome 15 of the mouse [11,12], but that region was very gene-rich and contained over 60 described genes. One gene of high interest was mouse Apobec3 (mA3), which had known anti-retroviral activity. In a recent collaboration with the Warner lab at the Gladstone Institute for Virology and Immunology, the mA3 gene was knocked out in Rfv3 resistant mice and extensive testing showed it to be the gene responsible for the Rfv3 phenotype [13]. The Rfv3 susceptible phenotype was found to be due to a splicing defect that resulted in the loss of exon 2 leading to translation of a protein defective in its antiviral activity. The mechanisms through which mA3 influences the antibody response are still under investigation.

CD4 Control of Chronic Infection

An effective immune response to FV infection requires a coordinated response involving many immune cell types, including CD4⁺ T cells. Selective CD4⁺ depletion experiments in chronic FV mice substantiated the importance of these cells in protective immunity, as relapse occurred in CD4-depleted mice (Hasenkrug J virol 72:6559 1998). And while CD4⁺ T cells can provide B cells and CD8⁺ T cells with immunological help, our studies suggest that their primary functions are IFN γ production and direct cytotoxic activity against virally infected cells. An FV-specific CD4⁺ T cell clone significantly reduced virus production in vitro by utilizing direct cytolytic activity and IFN γ -mediated inhibition (Iwashiro 2001 J virol 75, 52). Additionally, viral production was reduced when recombinant IFN γ or IFN γ -containing supernatants were applied in vitro. The importance of IFN γ and cytolysis is further demonstrated by mice genetically deficient in IFN γ , Fas or FasL, which fail to control FV infection (Dittmer J virol 75 654 2001 & Stromnes J virol 76:2225 2002 & Zelinsky Virol 330:365 2004). The necessity for CD4⁺ T cell effector function beyond providing help has also been demonstrated in LCMV and HIV (Jellison JI 2005 174:614, Norris J Virol 75, 9771-9 2001, Appay 2002 JI 168, 5954) and may be a useful population to target for viral immunotherapy.

CD8 Loss of Function

As mentioned, FV-specific CD4⁺ T cells are critical for the control of chronic infection. In striking contrast, depletion of the CD8⁺ T cell subset showed no significant protective role of these cells during the chronic phase. One possible explanation was viral mutation of the immunodominant H-2 D^b/GagL epitope (Chen J Virol 70, 7773 1996) but investigation showed only wild type sequence and that the virus was not utilizing latency as a means of escape (Dittmer Immunity). Furthermore, when FV-specific transgenic CD8⁺ T cells were adoptively transferred into chronic mice they were activated and proliferated, which suggests functional cognate-antigen recognition through the APC compartment (Dittmer Immunity). However, when these transgenic and FV-specific endogenous CD8⁺ T cells were further analyzed it was discovered they were dysfunctional in cytotoxic molecule production, showed little evidence of recent degranulation and were deficient in IFN γ (Zelinskyy J virol 2005 & Dittmer Immunity). Some of this dysfunction was even evident at the transcriptional level, suggesting a strong immunosuppressive influence on CD8⁺ T cell function during the chronic phase of FV infection. In contrast, FV-specific transgenic CD8⁺ T cells adoptively transferred into acute mice developed proper effector function, suggesting that the negative regulation was specific to the chronic phase of infection.

These studies on the loss of CD8⁺ T cell function investigated cells taken from the spleens of chronically infected mice. To determine whether the suppression was localized within the splenic lymphoid tissue or could also be found in non-lymphoid tissues, the phenotype and function of FV-specific CD8⁺ T cells were compared between spleens and livers of chronically infected mice. Interestingly, cells from the liver have the ability to produce IFN γ , granzyme B and show evidence of recent degranulation (manuscript in progress). In addition, the suppression induced by chronic FV infection was also shown to weaken responses to unrelated nonviral antigens (Iwashiro PNAS 2001 & Shelly JI 2006 176). Taken together, these data imply the loss of CD8⁺ T cell function is influenced more by the tissue location of the cell than by the specificity of that cell. Therefore, it was of great interest to explore the mechanism of suppression during chronic FV infection.

Tregs

Studies indicate retroviral infections typically produce a progressive immunosuppression, which can be partly responsible for viral persistence. In many instances pathogen-induced Tregs are one critical component of this immunosuppression. Tregs are a specialized subpopulation of CD4⁺ T cells that act to suppress immune responses, thereby maintaining immunological balance during the course of infections and self-tolerance on a continual basis. When the Treg response is untimely and overactive the virus can utilize this to its advantage to subvert the host immune response. The first indication Tregs were abnormally active in the FV model was chronically infected mice had a defective antitumor immune response, whereas naïve mice mounted a productive response that resulted in tumor elimination (Iwashiro PNAS 2001). This was particularly surprising because the chronic mice contained tumor-specific CD8⁺ T cells and should have mounted an accelerated response instead of a terminal course of tumor progression. These unexpected results were partially explained by T cell subset adoptive transfer experiments that demonstrated a CD4⁺ T cell-mediated suppression only when the CD4⁺ cells were taken from chronically infected mice. CD4⁺ T cells taken from naïve mice did not induce suppression in this tumor system.

Further analysis revealed that Tregs taken from mice chronically infected with FV suppressed virus-specific CD8⁺ T cell responses both in vitro and in vivo (Shelly JI 2006 & Dittmer 2004 & Zelinskyy 2005), suggesting that an overactive Treg compartment is at least partly responsible for the observed suppression of CD8⁺ T cells. Studies have further shown

that FV-induced Tregs require direct cell contact to suppress and the presence of APCs are not required (Shelly). Furthermore, Treg suppressive activity has been demonstrated in vivo in both the CD25+ and CD25- subsets, although in vitro suppression has only been demonstrated in the CD25+ population (Shelly JI 2006 176).

The interesting observation that FV-specific CD8+ T cells remain functional in the livers of chronically infected mice correlates with a lower proportion of Foxp3+ Tregs in the liver compared to the spleen (manuscript in progress). Furthermore, the liver Tregs in chronic mice appear less activated and express significantly less CD25 when compared to splenic Tregs. Consistent with these observations, FV infection in the liver appears to be successfully managed by the functional CD8+ T cell compartment. These observations suggest chronic retroviral infections might be successfully treated by therapeutic down-regulation of the Treg compartment, thereby restoring proper CD8+ T cell function. However, caution would be required as a recent report highlights the interesting observation that the induction of Tregs during herpes simplex virus (HSV) infection may actually be required for early protection (Lund 320 Science 08 & O'garra). Interestingly, mice selectively depleted of Tregs had enhanced susceptibility to HSV replication in vaginal epithelial cells regardless of the increased activity of proinflammatory cells, cytokines and chemokines in the lymphatic tissue. The interpretation of this report suggests that the Tregs are crucial in dampening the immune response in the lymph nodes, so that the response can be directed to the epithelial site of infection where early viral control is critical to prevent amplification of the infection. Thus, Tregs possess yet another layer of complexity in that they may partly control chemotaxis and the homing ability of immune mediators during infection. It is currently unknown if the induction of Tregs during chronic retroviral infection influences immune cell migration to or from lymphoid tissues. Clearly, the diversity of functions of Tregs must be fully appreciated and taken into account when designing immunotherapy strategies for retroviral infections.

Immunotherapy to Treat Chronic Infection

The finding that chronic FV infections and virus escape were associated with loss of CD8+ T cell function due to Treg-mediated suppression led us to investigate ways to counteract suppression. We first sought to determine if immunotherapy during acute infection could prevent Treg-mediated suppression and the establishment of chronic infection. We focused on the glucocorticoid-induced tumor necrosis factor receptor (GITR) because stimulation of GITR had been shown to eliminate suppressive function in Treg's [17] [18] and acted as a costimulatory molecule for effector T cells [19] [20]. Treatment of mice with an agonistic anti-GITR antibody during acute infection produced faster Th1 immune responses with significantly greater production of Th1 cytokines by both CD4+ and CD8+ T cells, significantly reduced virus loads and pathology, and partially prevented immunosuppression associated with chronic infection [21].

We were also interested in determining if we could reduce virus loads in mice with established chronic infections by re-stimulating virus-specific CD8+ T cells. Results had shown that virus-specific CD8+ T cells were present in chronically infected mice, but their effector functions had been shut down by regulatory T cells [22]. Chronically infected mice were treated with an agonistic antibody that triggered CD137 (4-1BB), a costimulatory molecule expressed on activated T cells. As with GITR, CD137 costimulation was reported to have effects on both Tregs [23] and effector T cells [24–28]. Our preliminary results indicated that CD137 signaling was even stronger than GITR signaling, however even with enhanced CD8+ T cell activation treated mice did not recover from FV infection.

In addition to simply treating mice with agonistic CD137-specific monoclonal antibodies, we also adoptively transferred FV-specific, TCR transgenic CD8⁺ T cells, both as a way to study the effects of therapy and as an additional therapeutic strategy. Results showed that CD8⁺ T cells adoptively transferred into chronically infected mice became rapidly suppressed unless the mice were treated with anti-CD137. Treatment with anti-CD137 rendered the adoptively transferred cells resistant to Treg-mediated suppression and stimulated dramatic reductions in chronic virus loads [29]. No direct effect on the Tregs was observed. Interestingly, anti-CD137 therapy did not work on already suppressed CD8⁺ T cells. Current studies are focused on immunotherapies that work without a requirement for adoptive T cell transfers, as this is a more practical therapeutic approach.

Vaccine Studies

Studies in the SIV model showed live attenuated viruses to be the most effective vaccines against retroviral infection, but it was not possible to establish the immunological correlates of protection in non-human primates (reviewed in [14]). Thus we decided to use live attenuated FV vaccination of genetically pure congenic mice to approach the problem of determining what types of immune mediators were involved in protection. Experiments in which single, double, or triple combinations of immune cell subsets were adoptively transferred from vaccinated mice to unvaccinated mice revealed a number of interesting findings [15]. By themselves, immune CD8⁺ T cells reduced acute virus loads by an order of magnitude, but the mice still became chronically infected. Adding immune CD4⁺ T cells or B cells did not significantly improve protection by CD8⁺ T cells, and in fact, no two subsets of immune lymphocytes protected the mice from chronic infection. However, any combination of two subsets of immune lymphocytes protected the mice from lethal disease. Most interestingly, adoptive transfer of all three immune subsets afforded complete protection from both clinical disease and the establishment of chronic infection. Subsequent studies showed that the primary role of immune B cells was the production of virus-specific antibodies [16]. The most effective antibodies in protection were neutralizing antibodies but this study once again highlighted the need for a complex immunological response against retroviral infection to obtain the best protection. Full protection was afforded only when antibodies were combined with vaccine-induced T cell responses.

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