

Immune Control and Prevention of Chronic Friend Retrovirus Infection

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1. ABSTRACT

T cells are critical to control acute infection of a host with retroviruses but they are usually unable to prevent the development of chronic infections. This review summarizes studies from the Friend virus mouse model that reveal some of the mechanisms by which T cells control chronic retroviral infection, and also reveal why these responses ultimately fail to fully eradicate infection. Also summarized are findings from vaccine studies demonstrating the immunological requirements for the prevention of chronic retroviral infection. The implications of these findings for chronic infections in humans are discussed.

2. INTRODUCTION

Infections with retroviruses such as Human Immunodeficiency Virus (HIV) and Human T-cell Leukemia Virus (HTLV) typically induce only mild to moderate symptoms during the acute phase because the immune system is able keep the infection under control

However, despite control of acute infection retroviruses utilize a number of escape mechanisms that allow them to resist complete eradication. Thus lifelong persistence is a hallmark of infection with these agents and therapies to eliminate chronic infections have been largely unsuccessful. One possible way to deal with chronic infections would be to activate the immune system in such a way that the virus would be unable to escape. Yet in order to develop rational immunotherapeutic approaches to cure chronic infections it is necessary to fully understand the fundamental immunological factors that are at play. To this end we have studied mice chronically infected with Friend retrovirus. These studies have revealed a mechanism of escape and maintenance of persistence that appears to be common among chronic infections of many types including viral, bacterial, parasitic and fungal infections (1,2). Either by direct or indirect means, chronic infectious agents are able to subvert the regulatory T cell system that normally prevents autoimmune diseases and immunopathology. Studies of mice chronically infected with Friend virus have

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revealed a complex balance of immune responses including two distinct CD4⁺ T cell subsets with opposing effects. A CD4⁺ T cell effector subset exerts the antiviral activity required to keep virus replication in check while a CD4⁺ regulatory subset suppresses the ability of CD8⁺ T cells to eliminate the infection. Deciphering the mechanisms by which both these subsets operate will allow us to develop ways to modulate their activity to the benefit of the host.

3. FRIEND VIRUS

FV is a complex of two viruses: Friend murine leukemia virus (F-MuLV), a replication competent helper virus that is nonpathogenic in adult mice; and spleen focus-forming virus (SFFV), a replication-defective virus that is the pathogenic component (3,4). SFFV cannot produce its own particles so it spreads by being packaged in F-MuLV-encoded particles produced in cells co-infected by both viruses. Pathology in susceptible adult mice is characterized by polyclonal proliferation of erythroid precursor cells resulting in massive splenomegaly. Splenomegaly is caused by a false proliferative signal induced by the binding of SFFV gp55 envelope glycoproteins to erythropoietin receptors on nucleated erythroid cells (5). In this way infection produces a greatly expanded population of actively dividing cells susceptible to FV infection. Eventually SFFV genomes integrate into two specific sites common to FV-induced erythroleukemias, Spi1 (6) and p53 (7). Insertional mutagenesis-mediated deregulation and over expression of the Spi-1 proto-oncogene combined with integration-induced inactivation of the p53 tumor suppressor gene results in fully malignant erythroleukemias. Resistant strains of mice are those that mount immune responses with sufficient speed and potency to prevent accumulation of these two transformation-associated events (8,9). However, even the most resistant strains of mice are never able to completely clear virus-infected cells (10).

4. CD4⁺ T-CELL-MEDIATED CONTROL OF CHRONIC INFECTION

CD4⁺ T cells are classically known as helper T cells that provide help for the immunological effectors, cytotoxic T lymphocytes and antibody-producing B cells. However, CD4⁺ T cell subsets also show direct antiviral activity in viral infections (11, 12, 13). In mice chronically infected with FV, depletion experiments showed that CD4⁺ T cells were critical for the control of chronic FV and prevention of disease recrudescence (14). The activity of the CD4⁺ T cells appeared to depend on direct antiviral activity rather than merely offering help to B cells or CD8⁺ T cells. First of all, relapse of disease in CD4-depleted mice did not correlate with loss of virus-neutralizing antibodies. Furthermore, it was unlikely that CD4⁺ T cells provided help for CD8⁺ T cells because CD8-depletion experiments showed no role for CD8⁺ T cells during chronic infection. This lack of CD8⁺ T cell effect was in stark contrast to their important role in initial recovery from acute infection (15.), and suggested that the chronic virus had escaped CD8⁺ T cell responses.

Consistent with a direct antiviral role for CD4⁺ T cells in controlling FV infections, *in vitro* studies showed that an FV-specific CD4⁺ T cell clone could significantly reduce FV production by infected cells (16). Both contact-dependent cytotoxicity and the secretion of interferon gamma (IFN-gamma) were involved in reduction of virus production. Fas/FasL-mediated lysis of infected target cells may be involved in *in vivo* control of chronic FV infections because mice deficient in either of these two molecules failed to control chronic FV infections (17). IFN-gamma from CD4⁺ T cells had several effects on virus control *in vitro* including enhancement of CD4⁺ T cell-mediated cytotoxicity and direct inhibition of infectious virus production (16). *In vitro* treatment of virus-producing cells with IFN-gamma-containing supernatants from activated FV-specific CD4⁺ T cells or with recombinant IFN-gamma resulted in a ten-fold decrease in the amount of infectious FV produced. A central role for IFN-gamma in the control of persistent FV infection was also shown in experiments with cytokine knockout mice. Mice with genetically inactivated IFN-gamma that were otherwise resistant to acute FV-induced disease were susceptible to late onset of erythroleukemia (18, 19). In contrast, similar experiments with mice deficient in the Th2 type cytokines interleukin (IL) IL-4, IL-5, IL-6 or IL-10 revealed that these cytokines were not important for controlling chronic FV infection (18, 20). Thus, IFN-gamma-producing CD4⁺ T cells with cytotoxic activity appeared to be the critical T cell subset that kept persistent FV in check (Figure 1).

Direct CD4⁺ T cell-mediated antiviral activity might be a common defense mechanism in persistent retroviral infections. Norris et al. (13) recently described HIV-specific CD4⁺ T cell clones that showed cytolytic activity and secreted IFN-gamma. The induction or activation of such cells by therapeutic vaccination or other means of immunostimulation might be a way to maintain control of chronic retroviral infections.

5. CD8⁺ T CELL DYSFUNCTION IN CHRONICALLY INFECTED MICE

As mentioned, the finding that depletion of CD8⁺ T cells had no effect on chronic virus suggested that the virus had escaped control by these important immunological effectors. Investigation into possible escape mechanisms such as virus latency, CTL epitope mutation, and interference of antigen presentation revealed that FV was utilizing a novel mechanism of escape (21). First of all, quantitative PCR experiments revealed that the virus was not latent. Although the percentage of infected cells was extremely low compared to acute infections, virus transcription on a per-provirus level was equivalent. Next, sequencing of the immunodominant H-2 D^b/GagL epitope (22) showed only wild type sequence, with no mutations in either the GagL sequence or the flanking regions (21). Furthermore it was shown that deletion of virus-specific CD8⁺ T cells had not occurred as has been shown in infections with lymphocytic choriomeningitis virus (23,24). Finally, the ability of antigen presenting cells (APC) to present FV antigen to FV-specific CD8⁺ T cells was analyzed. It was reasoned that if chronic virus were

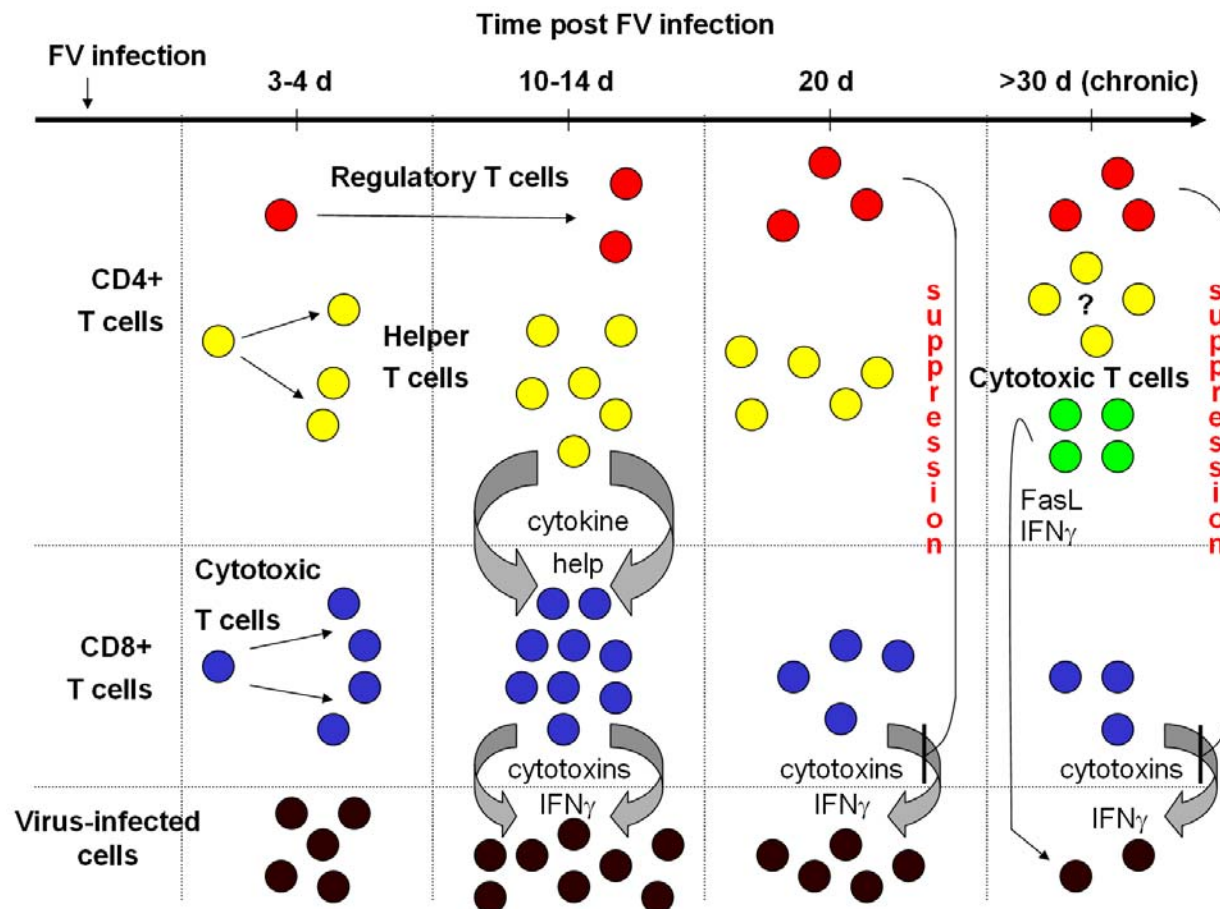


Figure 1. T cell responses during Friend retrovirus infection. During the first days of infection CD4⁺ helper T cells and CD8⁺ cytotoxic T cells are activated and start to proliferate. At the peak of virus replication (10 days post infection) CD8⁺ cytotoxic T cells produce interferon gamma (IFN- γ) and cytotoxic molecules (cytotoxins) and efficiently attack virus-infected cells. However, at this time point CD4⁺ regulatory T cells expand and suppress the antiviral activity of CD8⁺ T cells before the virus can be completely eliminated. During chronic infection, when CD8⁺ T cells are functionally impaired, CD4⁺ T cells that produce IFN- γ and mediate cytotoxic activity via Fas ligand keep chronic Friend virus in check. It is currently not known if these cytotoxic CD4⁺ T cells are a distinct T cell population or if they belong to the population of helper T cells, which is indicated by a "?".

interrupting antigen processing or presentation in any manner then adoptive transfer of naïve FV-specific CD8⁺ T cells into the chronically infected mice would result in a failure of the cells to activate and proliferate. For these experiments, CD8⁺ T cells from T cell receptor transgenic (TCR tg) mice specific for the FV GagL immunodominant epitope were used (25). Surprisingly, adoptively transferred CD8⁺ T cells recognized their cognate antigen and underwent rapid activation and proliferation following transfer into chronically infected mice. Thus there was no indication that chronic FV had escaped CD8⁺ T cell response by any of the previously described mechanisms.

Since the adoptively transferred CD8⁺ T cells expanded to very high numbers by two weeks post transfer, it was of interest to determine if these cells had any effect on chronic virus levels. While the same cells adoptively transferred into acutely infected mice could reduce infection levels by fifty fold, they had no effect on chronic

infection. Further analysis revealed that cells transferred into acutely infected mice developed effector function such as production of IFN- γ whereas cells transferred into chronically infected mice did not (Fig.1). Analyses of the endogenous population of virus-specific CD8⁺ T cells indicated that dysfunction was not peculiar to adoptively transferred cells, but could also be observed in the normal subset of FV-specific CD8⁺ T cells (26). These cells failed to produce three critical components of cytolytic granules, perforin, granzyme A, and granzyme B. Furthermore, they were shown to be defective in virus-specific cytolytic activity *in vivo*.

6. VIRUS-INDUCED CD4⁺ REGULATORY T CELLS

Clues regarding the reason for CD8⁺ T cell dysfunction in chronically infected mice came from studies in which these mice were found to be defective in their ability to mount CD8⁺ T cell-mediated responses to FV-

induced tumors (1). In those studies naïve mice could rapidly reject implants with FV-induced tumor cells but chronically infected mice could not. This was surprising because the FV-induced tumor cells expressed FV antigens and it was expected that the mice would mount anamnestic responses because of their prior exposure to FV antigens. Rather than possessing immunological memory though, the mice were immunosuppressed. *In vitro* experiments were done to analyze the ability of CD4⁺ and CD8⁺ T cells to mount allogeneic responses in mixed lymphocyte reactions. Results showed that CD8⁺ T cell responses were suppressed whenever the responder cells were mixed with CD4⁺ T cells from chronically infected mice. CD4⁺ T cells from naïve mice had no such effect. Thus chronic FV infection had induced a subset of immunosuppressive CD4⁺ T cells (regulatory T cells).

To determine if FV-induced regulatory T cells (Treg) were also suppressing the ability of chronically infected mice to mount effective CD8⁺ T cell-mediated anti-tumor responses, both CD4⁺ and TCR tg CD8⁺ subsets of T cells were purified from chronically infected mice and adoptively transferred into naïve mice. When the naïve mice were then challenged with FV-induced tumors or tumors unrelated to FV, those that had received CD8⁺ T cells could still reject the tumors, but those that had received CD4⁺ T cells could not. These experiments provided the first evidence demonstrating that a viral infection could induce regulatory T cells (1). The implication of these experiments was that the induction of Treg and the consequent suppression of CD8⁺ T cell responses allowed the virus to escape immunity, and to establish and maintain persistent infections.

To directly test the ability of FV-induced Treg to downregulate anti-viral CD8⁺ T cell function *in vivo*, differentially labeled CD4⁺ T cells and CD8⁺ T cells were co-transferred into recipient mice that were acutely infected. When virus-induced Treg from chronically infected mice were co-transferred with the CD8⁺ T cells, production of IFN- γ was significantly reduced in comparison to co-transfer with CD4⁺ T cells from naïve mice. Interestingly, co-transfer experiments using sub-fractions of Treg showed that both CD25 positive ("natural" Tregs) and CD25 negative subsets suppressed the IFN- γ production of CD8⁺ T cells. The CD25 positive subset of FV-induced Treg displayed an activated phenotype *in vivo* and suppressed antigen-stimulated FV-specific CD8⁺ T cell function *in vitro* (28). Suppression occurred directly *ex vivo* without any further stimulation. Interestingly, the upregulation of activation markers and the proliferation of the CD8⁺ T cells were not affected by FV-induced Treg. This finding recapitulated the type of suppression that was observed *in vivo* when FV-specific CD8⁺ T cells were adoptively transferred into chronically infected mice. Further studies on the mechanism of Treg-mediated suppression revealed several interesting features. Suppression was independent of APC's, was mediated by direct T cell-T cell interactions, and was not affected by the TCR specificity of the CD8⁺ T cells. Most interestingly, FV-induced Treg suppressed the function of CD8⁺ effector T cells that had been physiologically activated during acute

FV infection. The ability to suppress the effector function of activated CTL may be a very important activity of Treg *in vivo*. Recent evidence indicates that the normal role of Treg during the infectious process is limiting immunopathology by antiviral CD8⁺ T cells (Robertson & Hasenkrug, in press). Hyperactivity of CD8⁺ T cells can cause significant pathological damage if left unchecked, but suppression of CD8⁺ T cell function to prevent immunopathology must be tightly balanced with the need to allow CD8⁺ T cells to eliminate infected cells. Thus the timing and magnitude of the Treg response in relation to the CD8⁺ T cell response are critical factors in determining whether viruses are eliminated or become chronic. In acute FV infections Treg expansion peaks and the cells gain the ability to suppress CD8⁺ T cells at approximately 2 weeks post-infection, a time point when virus loads are still high (Zelinskyy et al., submitted). Concurrently CD8⁺ T cells begin to lose effector function. Thus the timing of suppression of the CD8⁺ T cell response appears too early to allow the CD8⁺ T cells to completely eradicate the infection (Fig.1). Similar kinetics of Treg cell expansion and CD8⁺ T cell dysfunction were found in the SIV model suggesting that Treg-induced immunosuppression might be a general phenomenon in chronic retroviral infections (29).

Do the findings of Treg-mediated suppression of CD8⁺ T cells in FV and SIV infections have relevance to chronic viral infections in humans? It has been known for some time that chronic HCV (30) and HIV infections (31,32,33) are associated with dysfunctional CD8⁺ T cells. While there may be multiple reasons for CD8⁺ T cell dysfunction, there is mounting evidence that regulatory T cells suppress CD8⁺ T cell responses in both HCV (34,35,36) and HIV infections (37,38,39). A direct correlation was found between levels of HIV-induced Treg and virus production in lymphoid tissue of infected patients (40). The accumulation of Tregs in HIV-infected lymph nodes appears to be due to an abnormal abundance of semimature dendritic cells that induce tolerance by causing T cells to adopt the phenotype of Treg (41). However, the role of virus-induced Treg in HIV infections is complex and these cells may also have beneficial impacts on the host as well. HIV infection causes hyperactivation of the immune response that results in immunopathology, and the degree of hyperactivation is predictive of progression to AIDS (42,43,44,45,46). It appears that while Treg contribute to virus persistence by suppressing immune responses that might eliminate the infection, they also help control HIV-induced hyperactivation-induced immunopathology. In fact, loss of Treg from the blood of HIV patients is an indicator of poor clinical outcome (39,47). A similarly complex situation exists in HCV infections where Treg-mediated suppression correlates with establishment of chronic infections, but once chronic infections are established Treg can prevent CD8⁺ T cell-mediated liver cirrhosis (48,49).

7. VACCINE PROTECTION AGAINST CHRONIC FV INFECTION

Since the most severe diseases associated with retroviral infections such as HIV are manifested during chronic rather than acute infection, protection against the

establishment of chronic infections is a requisite for an effective retroviral vaccine. Although an HIV vaccine that reduced viral loads during the acute phase could slow progression to disease, such a vaccine would be unlikely to provide long-term protection. Studies in the SIV model showed that the most effective vaccines were live attenuated viruses (50). Current live attenuated retroviruses are too dangerous to use in human because of their ability to persist and mutate to virulent forms (51,52,53). However, they are extremely interesting in terms of their ability to protect, and determining their mechanisms of protection could lead to rational vaccine designs of similar efficacy. Similar to SIV, live-attenuated FV provided sterilizing immunity whereas vaccines such as recombinant vaccinia viruses expressing FV genes protected against acute disease but not chronic infection (54, 55). To determine the role of different lymphocyte subsets in live attenuated vaccine protection against FV, different lymphocyte subpopulations from vaccinated mice were adoptively transferred to naïve mice, which were then challenged with pathogenic FV. Effects from passive transfer of FV-specific antibodies were also tested. Immune CD8⁺ T cells or virus-neutralizing antibodies alone mediated protection against acute disease, but not against the establishment of persistent infection. Additionally, no combination of any two lymphocyte subsets gave complete protection. In order to transfer protection against both acute disease and persistent infection, immune B cells as well as both CD4⁺ and CD8⁺ T cells were required (55,56). The requisite function of B cells was production of virus-neutralizing antibodies rather than activation of T cells (57). The combination of virus-neutralizing antibodies and T cells worked synergistically to provide greater protection than either component alone. Results from multiple studies indicated that the role of vaccine-elicited antibodies was to reduce the effective virus dose rather than totally prevent infection (57). It must be expected that vaccinated individuals will become infected. Therefore memory B cell and T cell responses should be in place to clear both free virus and virally-infected cells before the establishment of persistent infections can take place. These findings show that in principle it is possible to achieve complete vaccine protection against retroviral infection if immunological memory in all three major lymphocyte subsets is provoked.

8. CONCLUSION

The field of virus-induced Treg has great potential to produce new therapies to treat some of mankind's most serious diseases, but the field is still in its infancy and has revealed a previously unappreciated complexity in the immunological control of chronic infections. Without tight and careful control over how and when Treg activity is modulated to increase antiviral immunity, increased immunopathology could lead to dramatic and adverse consequences. Full comprehension of this complexity and development of immunomodulatory therapies will require continued study in animal models such as Friend virus in mice and SIV in non-human primates.

9. REFERENCES

1. Iwashiro, M., R. J. Messer, K. E. Peterson, I. M. Stromnes, T. Sugie & K. J. Hasenkrug: Immunosuppression by CD4⁺ regulatory T cells induced by chronic retroviral infection. *Proc Natl Acad Sci USA* 98, 9226-30 (2001)
2. Mills, K. H.: Regulatory T cells: friend or foe in immunity to infection? *Nat Rev Immunol* 4, 841-55 (2004)
3. Friend, C.: Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J Exp Med* 105, 307-318 (1957)
4. Kabat, D.: Molecular biology of Friend viral erythroleukemia. *Curr Top Microbiol Immunol* 148, 1-42 (1989)
5. Li, J. P., A. D. D'Andrea, H. F. Lodish & D. Baltimore: Activation of cell growth by binding of Friend spleen focus-forming virus gp55 glycoprotein to the erythropoietin receptor. *Nature* 343, 762-4 (1990)
6. Moreau-Gachelin, F., A. Tavittian & P. Tambourin: Spi-1 is a putative oncogene in virally induced murine erythroleukaemias. *Nature* 331, 277-80 (1988)
7. Munroe, D. G., J. W. Peacock & S. Benchimol: Inactivation of the cellular p53 gene is a common feature of Friend virus-induced erythroleukemia: relationship of inactivation to dominant transforming alleles. *Mol Cell Biol* 10, 3307-13 (1990)
8. Dittmer, U. & K. J. Hasenkrug: Cellular and molecular mechanisms of vaccine-induced protection against retroviral infections. *Curr Mol Med* 1, 431-6 (2001)
9. Hasenkrug, K. J. & U. Dittmer: The Role of CD4 and CD8 T Cells in Recovery and Protection from Retroviral Infection: Lessons from the Friend Virus Model. *Virology* 272, 244-249 (2000)
10. Hasenkrug, K. J.: Lymphocyte deficiencies increase susceptibility to Friend virus-induced erythroleukemia in Fv-2 genetically resistant mice. *J Virol* 73, 6468-73 (1999)
11. Jellison, E. R., S. K. Kim & R. M. Welsh: Cutting edge: MHC class II-restricted killing *in vivo* during viral infection. *J Immunol* 174, 614-8 (2005)
12. Norris, P. J., H. F. Moffett, O. O. Yang, D. E. Kaufmann, M. J. Clark, M. M. Addo & E. S. Rosenberg: Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. *J Virol* 78, 8844-51 (2004)
13. Norris, P. J., M. Sumaroka, C. Brander, H. F. Moffett, S. L. Boswell, T. Nguyen, Y. Sykulev, B. D. Walker & E. S. Rosenberg: Multiple effector functions mediated by human immunodeficiency virus-specific CD4(+) T-cell clones. *J Virol* 75, 9771-9 (2001)

14. Hasenkrug, K. J., D. M. Brooks & U. Dittmer: Critical role for CD4⁺ T cells in controlling retrovirus replication and spread in persistently infected mice. *J Virol* 72, 6559-6564 (1998)
15. Robertson, M. N., G. J. Spangrude, K. Hasenkrug, L. Perry, J. Nishio, K. Wehrly & B. Chesebro: Role and specificity of T-cell subsets in spontaneous recovery from Friend virus-induced leukemia in mice. *J Virol* 66, 3271-3277 (1992)
16. Iwashiro, M., K. Peterson, R. J. Messer, I. M. Stromnes & K. J. Hasenkrug: CD4(+) T cells and gamma interferon in the long-term control of persistent friend retrovirus infection. *J Virol* 75, 52-60 (2001)
17. Zelinskyy, G., S. Balkow, S. Schimmer, K. Schepers, M. M. Simon & U. Dittmer: Independent roles of perforin, granzymes, and Fas in the control of Friend retrovirus infection. *Virology* 330, 365-74 (2004)
18. Dittmer, U., K. E. Peterson, R. Messer, I. M. Stromnes, B. Race & K. J. Hasenkrug: Role of interleukin-4 (IL-4), IL-12, and gamma interferon in primary and vaccine-primed immune responses to Friend retrovirus infection. *J Virol* 75, 654-60 (2001)
19. Stromnes, I. M., U. Dittmer, T. N. Schumacher, K. Schepers, R. J. Messer, L. H. Evans, K. E. Peterson, B. Race & K. J. Hasenkrug: Temporal effects of gamma interferon deficiency on the course of Friend retrovirus infection in mice. *J Virol* 76, 2225-32 (2002)
20. Strestik, B. D., A. R. Olbrich, K. J. Hasenkrug & U. Dittmer: The role of IL-5, IL-6 and IL-10 in primary and vaccine-primed immune responses to infection with Friend retrovirus (Murine leukaemia virus). *J Gen Virol* 82, 1349-54 (2001)
21. Dittmer, U., H. He, R. J. Messer, S. Schimmer, A. R. Olbrich, C. Ohlen, P. D. Greenberg, I. M. Stromnes, M. Iwashiro, S. Sakaguchi, L. H. Evans, K. E. Peterson, G. Yang & K. J. Hasenkrug: Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. *Immunity* 20, 293-303 (2004)
22. Chen, W., H. Qin, B. Chesebro & M. A. Cheever: Identification of a gag-encoded cytotoxic T-lymphocyte epitope from FBL-3 leukemia shared by Friend, Moloney, and Rauscher murine leukemia virus-induced tumors. *J Virol* 70, 7773-82 (1996)
23. Doherty, P. C.: Immune exhaustion: driving virus-specific CD8⁺ T cells to death. *Trends Microbiol* 1, 207-9 (1993)
24. Moskophidis, D., F. Lechner, H. Pircher & R. M. Zinkernagel: Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* 362, 758-61 (1993)
25. Ohlen, C., M. Kalos, L. E. Cheng, A. C. Shur, D. J. Hong, B. D. Carson, N. C. Kokot, C. G. Lerner, B. D. Sather, E. S. Huseby & P. D. Greenberg: CD8(+) T cell tolerance to a tumor-associated antigen is maintained at the level of expansion rather than effector function. *J Exp Med* 195, 1407-18 (2002)
26. Zelinskyy, G., S. J. Robertson, S. Schimmer, R. J. Messer, K. J. Hasenkrug & U. Dittmer: CD8⁺ T-Cell Dysfunction due to Cytolytic Granule Deficiency in Persistent Friend Retrovirus Infection. *J Virol* 79, 10619-26 (2005)
27. Sakaguchi, S., N. Sakaguchi, J. Shimizu, S. Yamazaki, T. Sakihama, M. Itoh, Y. Kuniyasu, T. Nomura, M. Toda & T. Takahashi: Immunologic tolerance maintained by CD25⁺ CD4⁺ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 182, 18-32 (2001)
28. Robertson, S. J., R. J. Messer, A. B. Carmody & K. J. Hasenkrug: In Vitro Suppression of CD8⁺ T Cell Function by Friend Virus-Induced Regulatory T Cells. *J Immunol* 176, 3342-9 (2006)
29. Estes, J. D., Qingsheng, L., Reynolds, M. R., Wietgreffe, S., Duan, L., Schacker, T., Picker, L. J., Watkins, D. I., Lifson, J. D., Reilly, C., Carlis, J. & A. T. Haase: Premature Induction of an Immunosuppressive Regulatory T Cell Response during Acute Simian Immunodeficiency Virus Infection. *J Infect Dis* 193, 703-12 (2006)
30. Wedemeyer, H., X. S. He, M. Nascimbeni, A. R. Davis, H. B. Greenberg, J. H. Hoofnagle, T. J. Liang, H. Alter & B. Rehermann: Impaired effector function of hepatitis C virus-specific CD8⁺ T cells in chronic hepatitis C virus infection. *J Immunol* 169, 3447-58 (2002)
31. Andersson, J., S. Kinloch, A. Sonnerborg, J. Nilsson, T. E. Fehniger, A. L. Spetz, H. Behbahani, L. E. Goh, H. McDade, B. Gazzard, H. Stellbrink, D. Cooper & L. Perrin: Low levels of perforin expression in CD8⁺ T lymphocyte granules in lymphoid tissue during acute human immunodeficiency virus type 1 infection. *J Infect Dis* 185, 1355-8 (2002)
32. Appay, V., D. F. Nixon, S. M. Donahoe, G. M. Gillespie, T. Dong, A. King, G. S. Ogg, H. M. Spiegel, C. Conlon, C. A. Spina, D. V. Havlir, D. D. Richman, A. Waters, P. Easterbrook, A. J. McMichael & S. L. Rowland-Jones: HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. *J Exp Med* 192, 63-75 (2000)
33. Trimble, L. A. & J. Lieberman: Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3 zeta, the signaling chain of the T-cell receptor complex. *Blood* 91, 585-94 (1998)
34. Boettler, T., H. C. Spangenberg, C. Neumann-Haefelin, E. Panther, S. Urbani, C. Ferrari, H. E. Blum, F. von

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- Weizsacker & R. Thimme: T cells with a CD4⁺CD25⁺ regulatory phenotype suppress in vitro proliferation of virus-specific CD8⁺ T cells during chronic hepatitis C virus infection. *J Virol* 79, 7860-7 (2005)
35. Rushbrook, S. M., S. M. Ward, E. Unitt, S. L. Vowler, M. Lucas, P. Klenerman & G. J. Alexander: Regulatory T cells suppress in vitro proliferation of virus-specific CD8⁺ T cells during persistent hepatitis C virus infection. *J Virol* 79, 7852-9 (2005)
36. Sugimoto, K., F. Ikeda, J. Stadanlick, F. A. Nunes, H. J. Alter & K. M. Chang: Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 38, 1437-48 (2003)
37. Aandahl, E. M., J. Michaelsson, W. J. Moretto, F. M. Hecht & D. F. Nixon: Human CD4⁺ CD25⁺ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. *J Virol* 78, 2454-9 (2004)
38. Garba, M. L., C. D. Pilcher, A. L. Bingham, J. Eron & J. A. Frelinger: HIV antigens can induce TGF- β (1)-producing immunoregulatory CD8⁺ T cells. *J Immunol* 168, 2247-54 (2002)
39. Kinter, A. L., M. Hennessey, A. Bell, S. Kern, Y. Lin, M. Daucher, M. Planta, M. McGlaughlin, R. Jackson, S. F. Ziegler & A. S. Fauci: CD25⁺CD4⁺ regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4⁺ and CD8⁺ HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. *J Exp Med* 200, 331-43 (2004)
40. Andersson, J., A. Boasso, J. Nilsson, R. Zhang, N. J. Shire, S. Lindback, G. M. Shearer & C. A. Chougnet: Cutting edge: The prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. *J Immunol* 174, 3143-7 (2005)
41. Krathwohl, M. D., T. W. Schacker & J. L. Anderson: Abnormal Presence of Semimature Dendritic Cells That Induce Regulatory T Cells in HIV-Infected Subjects. *J Infect Dis* 193, 494-504 (2006)
42. Mahalingam, M., M. Peakman, E. T. Davies, A. Pozniak, T. J. McManus & D. Vergani: T cell activation and disease severity in HIV infection. *Clin Exp Immunol* 93, 337-43 (1993)
43. Hazenberg, M. D., S. A. Otto, B. H. van Benthem, M. T. Roos, R. A. Coutinho, J. M. Lange, D. Hamann, M. Prins & F. Miedema: Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *Aids* 17, 1881-8 (2003)
44. Deeks, S. G., C. M. Kitchen, L. Liu, H. Guo, R. Gascon, A. B. Narvaez, P. Hunt, J. N. Martin, J. O. Kahn, J. Levy, M. S. McGrath & F. M. Hecht: Immune activation set point during early HIV infection predicts subsequent CD4⁺ T-cell changes independent of viral load. *Blood* 104, 942-7 (2004)
45. van Asten, L., F. Danisman, S. A. Otto, J. A. Borghans, M. D. Hazenberg, R. A. Coutinho, M. Prins & F. Miedema: Pre-seroconversion immune status predicts the rate of CD4 T cell decline following HIV infection. *Aids* 18, 1885-93 (2004)
46. Sousa, A. E., J. Carneiro, M. Meier-Schellersheim, Z. Grossman & R. M. Victorino: CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol* 169, 3400-6 (2002)
47. Eggena, M. P., B. Barugahare, N. Jones, M. Okello, S. Mutalya, C. Kityo, P. Mugenyi & H. Cao: Depletion of regulatory T cells in HIV infection is associated with immune activation. *J Immunol* 174, 4407-14 (2005)
48. Longhi, M. S., Y. Ma, D. P. Bogdanos, P. Cheeseman, G. Mieli-Vergani & D. Vergani: Impairment of CD4⁺CD25⁺ regulatory T-cells in autoimmune liver disease. *J Hepatol* 41, 31-7 (2004)
49. Longhi, M. S., Y. Ma, R. R. Mitry, D. P. Bogdanos, M. Heneghan, P. Cheeseman, G. Mieli-Vergani & D. Vergani: Effect of CD4⁺ CD25⁺ regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis. *J Autoimmun* 25, 63-71 (2005)
50. Johnson, R. P. & R. C. Desrosiers: Protective immunity induced by live attenuated simian immunodeficiency virus. *Curr Opin Immunol* 10, 436-43 (1998)
51. Dittmer, U., T. Nisslein, W. Bodemer, H. Petry, U. Saueremann, C. Stahl-Hennig & G. Hunsmann: Cellular immune response of rhesus monkeys infected with a partially attenuated nef deletion mutant of the simian immunodeficiency virus. *Virology* 212, 392-7 (1995)
52. Baba, T. W., Y. S. Jeong, D. Pennick, R. Bronson, M. F. Greene & R. M. Ruprecht: Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques. *Science* 267, 1820-5 (1995)
53. Ruprecht, R. M., T. W. Baba & V. Liska: Attenuated HIV vaccine: caveats. *Science* 271, 1790-2 (1996)
54. Dittmer, U., D. M. Brooks & K. J. Hasenkrug: Protection against establishment of retroviral persistence by vaccination with a live attenuated virus. *J Virol* 73, 3753-3757 (1999)
55. Dittmer, U. & K. J. Hasenkrug: Different immunological requirements for protection against acute versus persistent Friend retrovirus infections. *Virology* 272, 177-182 (2000)
56. Dittmer, U., D. M. Brooks & K. J. Hasenkrug: Requirement for multiple lymphocyte subsets in protection

T cell responses in chronic Friend virus infection

against retroviral infection by a live attenuated vaccine.
Nature Med 5, 189-193 (1999)

57. Messer, R. J., U. Dittmer, K. E. Peterson & K. J. Hasenkrug: Essential role for virus-neutralizing antibodies in sterilizing immunity against Friend retrovirus infection.
Proc Natl Acad Sci U S A 101, 12260-5 (2004)

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