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Cytokine profiles during experimental Chagas' disease

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Abstract

People infected with *Trypanosoma cruzi* remain so for life, yet only 30-40% of these individuals develop characteristic chagasic cardiomyopathies. Similarly, when infected with the Brazilian strain of *T. cruzi*, DBA/2 mice develop severe cardiac damage while B10.D2 mice do not. To better understand the immunological parameters that may be involved in the disease process, we have used this murine model (DBA/2 vs B10.D2) and compared the changes in cytokine production during the course of infection with *T. cruzi*. Concanavalin A (Con A) stimulation of spleen cells harvested during the acute phase (day 30) resulted in similarly high levels of IFN-γ in both mouse strains. However, the amount of IFN-γ in supernatants from cultures of B10.D2 spleen cells initiated during the chronic phase (day 72) was at subacute levels, whereas secretion by chronic DBA/2 spleen cells remained high. In addition, Con A-stimulated spleen cells from acute DBA/2 mice produced approximately twice as much IL-10 and significantly more IL-4 than cells from B10.D2 mice. IL-4 secretion remained low by cells from chronic B10.D2 mice, but when using cells from chronic DBA/2 mice, levels continued to increase beyond the already high levels secreted by cells harvested during the acute phase. Proliferative responses to Con A stimulation by spleen cells from DBA/2 mice were significantly higher than those from B10.D2 mice in both the acute and chronic phases. These data suggest that enhanced responses in DBA/2 mice, which may be related to a higher parasite burden, a lack of down-regulation, and/or the onset of autoimmune phenomena, correlate with the more severe cardiomyopathy seen in pathopermissive mice.

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- IL-4

Trypanosoma cruzi is an obligate intracellular protozoan parasite of humans and other mammals and is the causative agent of *Chagas'* disease. During the acute phase of infection parasites are detectable in the blood, although the level of parasitemia is often low. The subsequent chronic phase is characterized by a decrease in the number of circulating parasites to levels that are usually not detectable, even though the host remains infected for life. The host's immune response also changes as the infection progresses from the acute to the chronic phase and is likely to be related to major shifts in cytokine production.

Using a comparative genetic model, we have previously shown that both the levels and the kinetics of cytokine production differ in strains of mice that differ in their ability to survive acute infection, suggesting

their importance in resistance (1). For example, we demonstrated that antigen-stimulated spleen cells from mice that express the resistant H-2^q MHC haplotype produced significantly more interferon-gamma (IFN-γ) than cells from mice that share the susceptible H-2^k haplotype. However, spleen cells from susceptible and resistant mice produced similar levels of IFN-γ when stimulated with concanavalin A (Con A), suggesting that the potential to produce this critical cytokine is similar in both resistant and susceptible strains, but that parasite-induced regulatory events down-regulate production in the susceptible mice. This may be related to the secretion of IL-10, which we found to be inversely correlated with IFN-γ levels throughout the course of acute infection. However, we found this inverse correlation of cytokine levels in supernatants from Con A-stimulated spleen cells from both resistant and susceptible strains. In addition, we found that cytokine production by lymph node cells differs from production by splenocytes. These results support those of others (2) who demonstrated that cytokine-producing cells are compartmentalized in different lymphoid organs during acute T. cruzi infection and further emphasize the complexity of immunoregulation during this disease.

More recently, we have examined immunological phenomena during both the acute and the chronic phase of T. cruzi infection. In these studies we compared in vitro cytokine production and changes in cell phenotype (Morato MJF, Freeman GL, Colley DG and Powell MR, unpublished data) in DBA/2 and B10.D2 mice after infection with the Brazilian strain of T. cruzi. DBA/2 mice develop high parasitemia during the acute phase and subsequently develop severe cardiomyopathy, and are termed pathopermissive (3). Conversely, B10.D2 do not develop high levels of circulating parasites or severe cardiac damage and are thus termed pathoresistant. We measured the levels of IFN-γ, IL-4, and IL-10 produced by spleen cells from each of these strains after stimulation in vitro. Studies during the acute phase were done using cells from mice infected for 21 or 30 days. In the chronic phase, we harvested cells from mice infected for 72-75 days, a time when differences in the degree of cardiac pathology between the two strains is demonstrable.

Splenocytes from both strains of mice infected for 21 days, when stimulated in vitro with Con A, secreted significantly higher levels of IFN-γ (>80 U/ml) compared to non-infected controls. Thirty days after infection, the levels secreted by spleen cells from both strains of mice were also similar, but had increased dramatically to >300 U/ml. However, during the chronic phase (72 days after infection) the amount of IFN-γ secreted by cells from pathoresistant mice had declined to lower levels than those found in the acute phase (<50 U/ml), whereas the quantity secreted by pathopermissive mice was sustained at >300 U/ml. Since at this time point (day 72) the numbers of circulating parasites have reached essentially undetectable levels in the blood of both strains of mice, these results suggest that the continuing inflammatory response may be related to a lack of down-regulation in pathopermissive mice, that there are increased levels of parasites sequestered in the solid tissues, and/or that autoimmune mechanisms are responsible for stimulating ongoing unchecked immunological, and potentially immunopathological, phenomena.

Con A stimulation of spleen cells prepared from both mouse strains during the acute phase (day 30) also resulted in enhanced IL-10 secretion. These results are consistent with other studies that have shown concurrent secretion of both inflammatory (TH1) and anti-inflammatory (TH2) cytokines during T. cruzi infection (4-7), including an analysis of cytokine mRNA expression in hearts from B10.D2 and DBA/2 mice (Powell MR, Morgan JM and Colley DG, unpublished data). Moreover, it should be

noted that the levels of *IL-10* detected in culture supernatants from Con *A*-stimulated *DBA/2* spleen cells *(day 30)* contained twice as much *IL-10* compared to their pathoresistant *B10.D2* counterparts. Yet the levels of *IFN-γ*, which are known to usually be inversely related to *IL-10 (7-9)*, were similar in cultures from the two strains at this time during the acute phase. *IL-10* production by cells from both strains of mice diminished to near control levels in cultures prepared on day *72* after infection, but cells from chronic *DBA/2* mice continued to secrete *IFN-γ* while cells from *B10.D2* mice did not *(*see above*)*, suggesting that different pathways of cytokine regulation may be occurring in these two strains of mice. This is further supported by the fact that spleen cells from *DBA/2* mice harvested *30* days after infection secrete little *IL-10* upon in vitro stimulation with *LPS*, whereas *B10.D2* mice secrete copious quantities under the same conditions. This result indicates that, if *IL-10* plays a role in cardiac pathogenesis *(*or the prevention thereof*)* in these mice, it may be derived from different sources.

Stimulation of spleen cells from infected *DBA/2* mice with *Con A* also resulted in significantly enhanced secretion of *IL-4*. Levels of *>300* pg/ml were found in supernatants using cells from mice infected for *30* days, which increased to *>800* pg/ml when cells from mice infected for *72-75* days were used. Spleen cells from *B10.D2* mice did not secrete *IL-4*, regardless of the time point after infection when the cells were harvested. In addition, proliferative responses, measured by *[*^{3}*H]*-thymidine incorporation after *Con A* stimulation of spleen cells from *DBA/2* mice, were elevated on day *30 (E/C = 12)* and even higher on day *75 (E/C = 57)*, while proliferation of *B10.D2* spleen cells was low *(E/C<3)* throughout the course of infection.

Taken together, these data suggest that a vigorous immune response in pathopermissive mice, not seen in the pathoresistant strain, may contribute to the severe pathogenesis found in these animals. Such an over-zealous response may be related to the lack of immunoregulation, a higher level of parasite burden *(3)*, and/or the onset of autoimmune phenomena *(10)*. Further study of this model should contribute to a better understanding of which of these mechanisms are related to the development and/or prevention of chronic chagasic cardiomyopathy.

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