Resistance to *Leishmania major* in Mice

The genetic determination of resistance to *Leishmania major* in mice is a topic of interest to many immunologists attempting to link the immunological mechanism with the responsible genes. In this respect, this experimental system is one of the paradigms for genetics of disease susceptibility. Mehmet L. Güler *et al.* (*1*) question the hypothesis that production of interleukin-4 (IL-4) with subsequent generation of a T helper cell type 2 (T_H2) response is the cause of susceptibility of BALB/c mice to *L. major* infection. Their data show that the susceptible BALB/c mice, but not the resistant B10.D2 mice, exhibit a diminished induction of interferon γ (IFN-γ) by IL-12 treatment, indicating a deficient T_H1 response, whereas the IL-4 response is the same in both strains. This deficit in the T_H1 response in BALB/c mice is linked to a region of chromosome 11 comprising IL-4, IRF-1, and several other immunologically relevant loci. Güler *et al.* propose that their findings are relevant for the host response to *L. major* infection because of the linkage between this chromosomal region and susceptibility to *L. major* suggested by Roberts *et al.* (*2*). They propose, therefore, that the resistance to *L. major* is based on maintenance of the IL-12 pathway and not on differential regulation of IL-4 production.

The genetic data argue against this simple model. We analyzed the genetic differences in T cell activation and resistance to *L. major* between the susceptible strain BALB/cHeA and the resistant strain STS/A, using the series of 20 homozygous CcS/Dem recombinant congenic (RC) strains. Each of the CcS/Dem strains carries a different random set of approximately 12.5% of the genes of the STS/A strain on the genetic background of the BALB/cHeA strain. Consequently, the individual loci involved in control of a multigenic trait were separated into different CcS/Dem strains, where they could be studied one by one (*3*). Our testing of susceptibility to *L. major* in the 20 CcS/Dem strains indicates that it is multigenically controlled (*4*). Moreover, the most resistant RC strain, CcS-5, received the whole central and telomeric part of chromosome 11 (including the IL-4 locus and the other linked immunologically relevant loci) from the susceptible parent BALB/c (*5*), indicating that this region cannot be the major factor determining susceptibility of BALB/c mice.

Even in the case that this region of chromosome 11 has some effect on *L. major* susceptibility, it has by no means been prov-

en that this effect is based on the IL-12 response. We found that the quantitative differences in proliferative T cell response to TCR-mediated activation is also closely linked to this region (*6*). This indicates that responsiveness to a variety of cytokines driving T cell proliferation (including IL-2, which we have tested separately) is controlled by this genetic region. Therefore, Güler *et al.* cannot pinpoint the control of susceptibility to *L. major* to IL-12 responsiveness only, because other cytokines and receptors may be involved as well. Thus, the claim that the genetic deficit of T_H1 response is the major cause of susceptibility to *L. major* cannot be maintained on the basis of the currently available data. Taking into consideration that the susceptibility of BALB/c mice is determined by several genes, it is unlikely that the reported difference is based on a single step of the immune response.

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Response: We agree with the comments of Demant *et al.* Resistance to *L. major* is likely a polygenic phenomenon and may be achieved through different mechanisms in different strains of mice. For example, resistance shown by B10.D2 mice may be qualitatively different from that of C3H mice (*1*, *2*). Both strains are "resistant," but resistance by B10.D2 is not associated with

early IL-12 production and is independent of NK cell participation (*1*). In contrast, resistance by C3H is associated with early IL-12 production and strongly relies on the NK responses (*2*, *3*). Thus, the different modes of resistance exhibited by various strains may rely in part on multiple genetic loci.

We analyzed T_H1 and T_H2 development in vitro for B10.D2 and BALB/c (*4*) and found a single locus controlling this difference on mouse chromosome 11 (*5*). This locus controls T_H1 development for T cells activated in vitro without adjuvants.

Demont *et al.* analyzed different strains (STS/A and BALB/c) and found that resistance in STS/A was not dependent on the same region of chromosome 11 that controls T_H1 and T_H2 development in B10.D2 mice. Their finding is consistent with multiple components of resistance and with different strains using separate mechanisms to control *L. major* infection. We found that *L. major* "resistant" DBA/J mice showed an in vitro phenotype similar to BALB/c (T_H2); thus, the mechanism of resistance to this pathogen in DBA/J and C57B1/6 may not be identical (*6*). Similarly, B10.D2 and STS/A may achieve resistance through nonidentical mechanisms.

P. Scott recently found a difference in the mechanism of resistance for C57B1/6 and DBA/J (*7*). C57BL/6 mice were able to overcome *L. major* infection even after initial in vivo neutralization of IL-12, whereas DBA were not. This observation suggests that the increased maintenance of IL-12 responsiveness we found in B10.D2 mice could provide an extra layer of protection in that strain, which if absent (as in DBA/J), could lead to greater dependence on early IL-12 synthesis.

In this way, this locus acts as a disease modifier. The same region of chromosome 11 was recently found to be a modifier in another T_H1- and T_H2-dependent disease, experimental allergic encephalomyelitis (EAE) (*8*), controlling its severity, but not its initiation. We have proposed that this locus is a modifier of immune response (T_H1 and T_H2), and could contribute to the robust *L. major* resistance of C57BL/6 and B10.D2 strains, and also to the expression or severity of several diseases involving T_H1 and T_H2 type responses, such as atopy and autoimmunity. We do not however, as some may have interpreted, suggest that this is the only locus involved in *L. major* susceptibility or in other diseases.

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Role of β-Chemokines in Suppressing HIV Replication

Fiorenza Cocchi et al. (1) found that three different β-chemokines (RANTES, MIP-1α, and MIP-1β) produced by CD8$^+$ T lymphocytes suppress human immunodeficiency virus (HIV) replication in peripheral blood mononuclear cells (PBMC). Moreover, neutralizing antibodies to all three chemokines eliminate the activity against HIV detected in CD8$^+$ cell supernatants (1). They conclude that these chemokines are responsible for the CD8$^+$ cell anti-HIV activity described in our studies (2–4).

During our attempts to identify CD8$^+$ cell antiviral factors (CAFs) that could mediate CD8$^+$ cell anti-HIV activity, several cytokines—including the interferons α and β, the chemokine IL-8, TGF-β, TNF-α, and the β-chemokines reported by Cocchi et al.—were identified as having antiviral activity (3–5) (see below). None of these cytokines, however, has been present in consistent or sufficient amounts to be CAF.

In addition, neutralizing antibodies to these cytokines have not affected the extent of the anti-HIV activity that we have detected in CD8$^+$ cell culture fluids (3–5).

In our evaluation of the β-chemokines, we found that the concentrations of RANTES, MIP-1α, and MIP-1β in CD8$^+$ cell supernatants [as measured by enzyme-linked immunosorbent assay (ELISA)] did not correlate with the anti-HIV activity detected in our assays (Fig. 1). Culture fluids with high anti-HIV activity (6) had concentrations of these chemokines from 0.1 to 4 ng/ml, and culture fluids lacking antiviral activity showed similar concentrations. Moreover, none of these three chemokines, even when used together, inhibited HIV-1$_{SF2}$ replication in purified CD4$^+$ cells at the concentrations found in supernatants of CD8$^+$ cells nor at the concentrations reported by Cocchi et al. (1) (Fig. 2A). This virus is one used in our measurement of CAF antiviral activity (3, 4, 6, 7).

When these chemokines were used with a variety of freshly isolated viruses, different sensitivities were noted (see examples, Fig. 2, B to D). Some were highly sensitive to the chemokines (for example, SV), some were resistant, and others showed an intermediate pattern. Cell antiviral factors show antiviral activity against all these viruses. As expected, a mixture of neutralizing antibodies to the three β-chemokines, at quantities similar to those cited (1), did not block the antiviral activity of CD8$^+$ cell supernatants against acute HIV-1$_{SF2}$ repli-

Fig. 1. RANTES, MIP-1α, and MIP-1β in CD8$^+$ cell culture fluids that were positive (hatched bars) or negative (open bars) for CAF antiviral activity. Chemokine concentrations in randomly selected CD8$^+$ cell culture fluids were measured by ELISA (Quantikine kits, R&D Systems, Minneapolis, Minnesota) in duplicate according to the manufacturer's instructions. CAF activity of each fluid was previously determined as described (4, 6, 7). Data shown are representative of nine CAF-positive and -negative fluids. Fluids were collected on days 7 through 12 from cultured CD8$^+$ cells from asymptomatic HIV-1–infected individuals. This time period is optimal for CAF production in vitro (4).
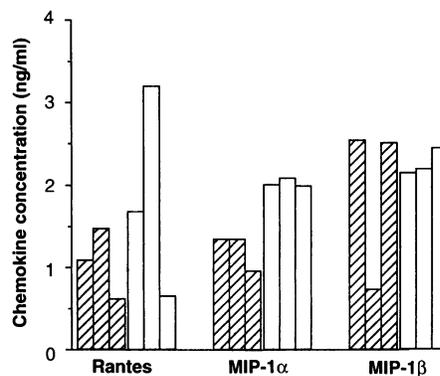

Fig. 2. Effect of recombinant chemokines on HIV-1 replication in CD4$^+$ cells. (**A**) CD4$^+$ cells acutely infected with HIV-1$_{SF2}$ (4). (**B** through **D**) Primary isolates of HIV-1 obtained from asymptomatic long-term survivors (B) SV, (C) NB, or (D) EM were cultured in triplicate in the presence of a mixture of the recombinant human chemokines RANTES, MIP-1α, and MIP-1β (R&D Systems). The concentrations of the chemokines tested were 500 ng/ml (●), 50 ng/ml (▲), 5 ng/ml (♦), and no chemokine added (■). The cultures were passed every 2 days and replenished with fresh chemokines at each passage. Reverse transcriptase (RT) activity was measured in culture fluids from the indicated time-points as described (4).
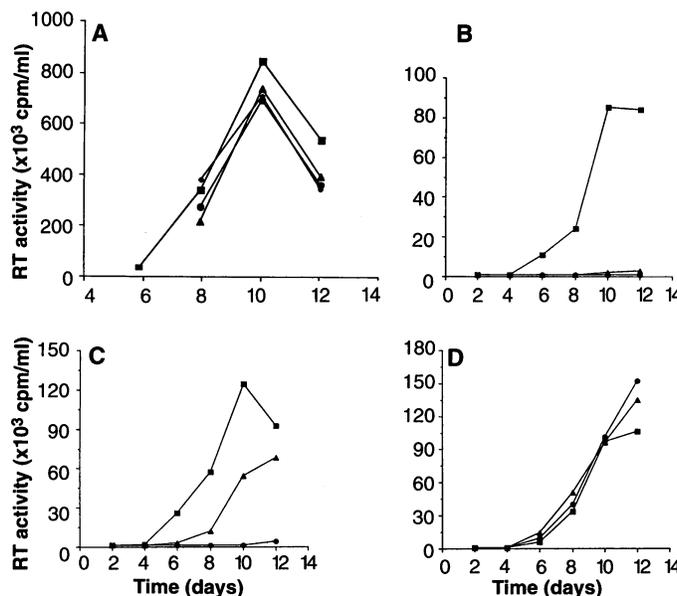

Table 1. Effect of neutralizing antibodies to β-chemokine on the ability of CAF-containing fluids to suppress HIV-1 replication in CD4$^+$ lymphocytes. A 50% dilution of CAF-containing culture fluid, or the medium control fluid, was left untreated or pretreated with control antibodies (Ab) or a mixture of antibodies specific for the β-chemokines for 40 min at room temperature before addition to HIV-1$_{SF2}$–infected CD4$^+$ lymphocytes. Control antibody was nonspecific goat polyclonal (R&D Systems); anti-chemokine antibody mix consisted of goat polyclonal neutralizing antibodies specific to the human chemokines, RANTES (100 μg/ml), MIP-1α (50 μg/ml), and MIP-1β (100 μg/ml) (R&D Systems). Reverse transcriptase (RT) activity shown indicates the average peak of HIV-1$_{SF2}$ replication (at day 10) in triplicate cultures. Fluids contained β-chemokine levels similar to those shown in Fig. 1. Culture fluids receiving the antibodies to chemokine showed a complete elimination of the chemokines as detected by ELISA (5).

Antibody treatment	RT activity (×1000 cpm/ml)	
	CAF-containing fluid	Control fluid
Untreated	1952	942
Control Ab	1740	923
Chemokine-Ab mix	1881	903

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