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Role of Gamma-Delta T Cells in Murine *Chlamydia trachomatis* Infection

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The role of gamma-delta T cells in host resistance to *Chlamydia trachomatis* was characterized by using a murine model of pneumonia caused by the mouse pneumonitis agent (MoPn), murine *C. trachomatis*. At days 3 and 7 after infection, gamma-delta T-cell-deficient knockout mice had significantly higher levels of MoPn in the lungs than did immunologically intact controls. At day 20, paradoxically, gamma-delta T-cell-deficient mice were more resistant to MoPn than were controls. This increased resistance was not due to an increased production of toxic cytokines or interleukin-10 in controls on that day. Gamma-delta T cells play a role in protection early in MoPn infection, but they may be deleterious later in infection, as has been observed in models of salmonella and trypanosome infection.

Gamma-delta T-cell receptor T cells have played a positive role in host defense against a variety of pathogens, in part through gamma interferon (IFN-γ) secretion by gamma-delta intraepithelial lymphocytes (7, 14, 17, 23). Interleukin-1 (IL-1) and IL-12 can activate gamma-delta T cells for production of IFN-γ, which subsequently directs the immune system towards a Th1 response (18) that may be beneficial in host defense.

A gamma-delta T-cell-mediated suppressor function has also been described previously (3). Gamma-delta T cells in experimental infection can express mRNA for downregulatory cytokines such as IL-4 and IL-10 and thus have the potential to suppress host defense mechanisms (4). Therefore, the possibility of a role for gamma-delta T cells in stimulating a protective Th1 response early in infection with a potential downregulatory role later is of importance for studies investigating the function of gamma-delta T cells in host defense.

It is of interest that the lack of gamma-delta T cells has had an apparent beneficial effect in at least two infectious disease models. With *Salmonella choleraesuis* infection, the survival of gamma-delta T-cell-deficient mice was improved compared with that of controls in association with lower levels of tumor necrosis factor alpha in serum and thus perhaps fewer toxicity-related deaths (although the levels of salmonella infection in tissue were not determined) (5). For chronic infection with Chagas' disease (although the numbers of circulating parasites in gamma-delta T-cell-deficient animals did not differ from those of controls, and the levels of mRNA for IFN-γ were actually higher in gamma-delta T-cell-deficient mice at baseline and early in infection), the extent of apparently deleterious inflammation was decreased in heart tissues in the gamma-delta T-cell-deficient animals and survival was improved (9). In this instance, gamma-delta T cells did not play a pivotal role in clearance of the organism but appeared to be involved in deleterious tissue pathology late in infection.

The role of gamma-delta T cells in chlamydial infection has not been explored. A variety of data strongly imply an important role for IFN-γ and Th1 responses in protection against chlamydial infection (2, 8). Therefore, gamma-delta T-cell function early in chlamydial infection could play an important, beneficial role by directing host defense towards a Th1 response.

Gamma-delta T lymphocytes are also of potential interest in chlamydial infection because a minority of gamma-delta T cells respond to peptides from highly evolutionarily conserved heat shock proteins (HSP) (6). While it is not clear that HSP stimulate only gamma-delta T cells to T-cell receptor-dependent reactivity, the facts that HSP can stimulate gamma-delta T cells and that gamma-delta T cells may play a role in the expression of HSP in host macrophages (14) are potentially important in chlamydial infection. Although controversial, there are substantial data indicating that a 57-kDa chlamydial homolog of the HSP GroEL is important in inducing potentially deleterious hypersensitivity responses during chlamydial infection that can lead to scarring and infertility (13, 19). This HSP may be an important antigen in inducing these effects because of its apparent ability to persist in tissue infected with chlamydiae (1). A potential response of gamma-delta T cells to HSP could thus play an important role both in initiating a protective Th1 host defense during chlamydial infection and in inducing a potentially deleterious autoimmune-based immunopathological response (reviewed in reference 16). Thus, the potential positive or negative roles of gamma-delta T cells in host defense and immunopathogenesis in chlamydial infection are important.

The studies reported here employing our model of murine pneumonia caused by the mouse pneumonitis agent (MoPn; murine *Chlamydia trachomatis*) (11) begin the investigation of the role of gamma-delta T cells in chlamydial infection by examining their role in host defense at various times during infection. The results obtained are consistent with those observed in other infectious disease models in that gamma-delta T cells apparently play a dual role in host defense in this infection, with both beneficial and deleterious effects.

Our initial studies involving the role of gamma-delta T cells in host defense employed nude athymic and heterozygous mice with a BALB/c background from our animal colony infected with various doses of MoPn and treated in vivo with an antibody to gamma-delta T cells (either GL3 or UC7-13D5). While a trend to greater susceptibility to MoPn was observed in T-cell-depleted mice, the results, in general, were variable and

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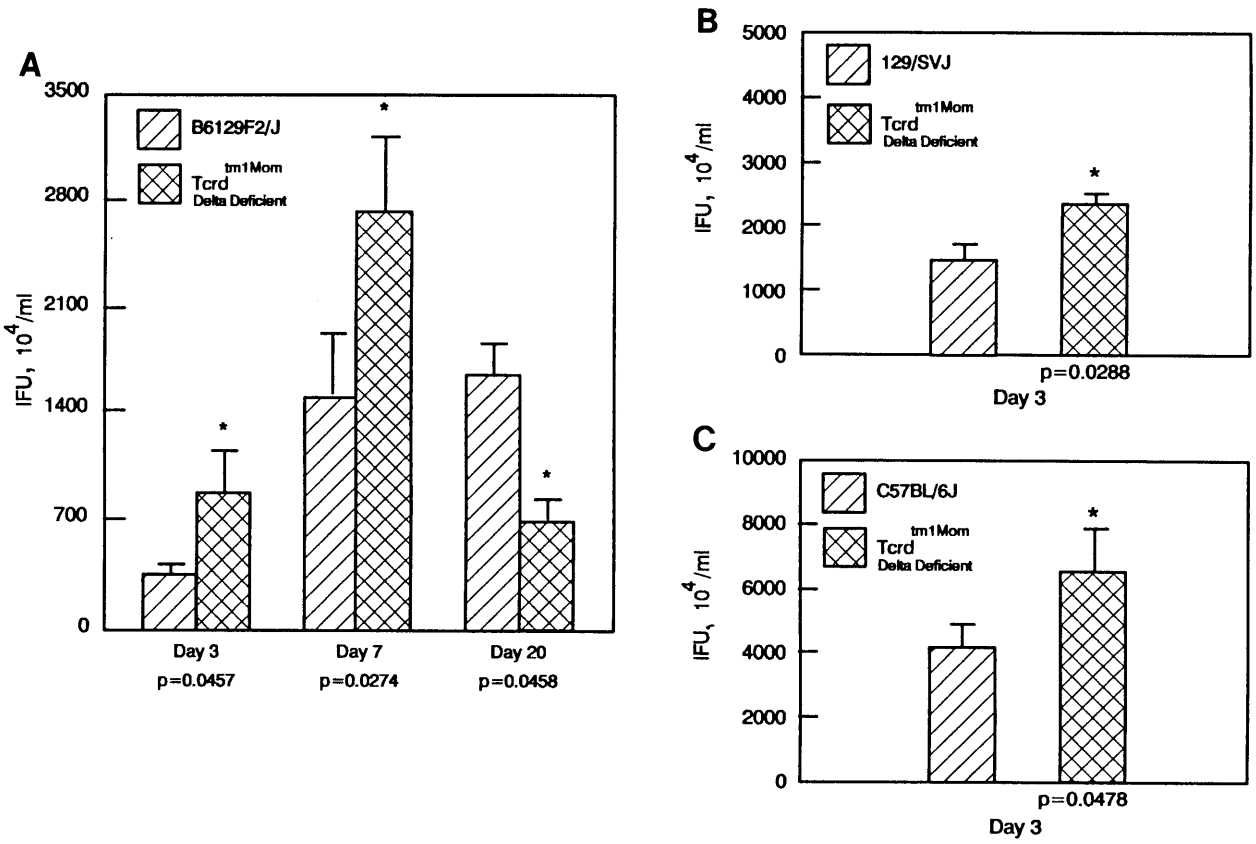

FIG. 1. (A) Chlamydial levels in lung tissues of B6129F2/J control and Tcrd^{tm1Mom} gamma-delta T-cell-deficient mice after infection with MoPn intranasally on day 0. The results are representative of a combination of three similar experiments with a total of 10 to 12 mice per group on days 3 and 7 and 7 to 9 mice per group on day 20. The data for two mice in the B6129F2/J group which died prior to sacrifice on day 20 were not included. The results are protein determinations, expressed as IFU equivalents, by ELISA. (B and C) Chlamydial levels in lung tissues of B129SvJ or C57BL/6J parental strain mice and gamma-delta T-cell-deficient Tcrd^{tm1Mom} mice on day 3 postinfection in groups of three to five animals each. The results for separate experiments with slightly different MoPn inocula are shown in panels B and C.

had borderline statistical significance for the MoPn doses examined, with only partial gamma-delta T-cell depletion observed in the lungs (unpublished data).

To assure adequate and consistent depletion of gamma-delta T-cell function, the mice used in these studies were STOCK Tcrd^{tm1Mom} with a T-cell receptor delta-chain-targeted mutation, produced by Peter Mombaerts in the laboratory of Susumu Tonegawa, which inhibits expression of the gamma-delta T-cell receptor in adult lymphoid or epithelial organs (12). The genetic background of the mice is mixed (B129×C57BL/6) and is not homogeneous. Controls were B6129F2/J mice. All mice were obtained from Jackson Laboratories.

Mice were infected intranasally with 1×10^4 to 5×10^4 inclusion-forming units (IFU) of MoPn, as in our prior studies (11). Infection in the lung was determined by antigen enzyme-linked immunosorbent assay (ELISA) as described in our previous study (11). The results of this assay have correlated very well with those of quantitative culture (correlation coefficient of 0.8). The results are expressed as equivalent inclusion-forming units. The percentages of gamma-delta T cells within whole lung and spleen tissues 3 days after MoPn infection were determined by flow cytometry. Single-cell suspensions of lung and spleen tissues were prepared by mechanical dispersion by first mincing the tissues with scissors and then expressing the cells through a 70-μm nylon mesh (Becton Dickinson Labware, Franklin Lakes, N.J.). Erythrocytes were lysed with 0.17 M Tris-NH_4Cl, and the cells were washed twice and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 1% bovine serum albumin (Sigma, St. Louis, Mo.). Single-cell suspensions of tissue from individual mice were stained with both anti-mouse gamma-delta monoclonal antibody conjugated to fluorescein isothiocyanate and anti-mouse CD3 conjugated to phycoerythrin (Caltag, San Francisco, Calif.), as previously described (10). Flow cytometry was performed with freshly stained cells by using a fluorescence-activated cell sorting (FACS) analyzer and Lysys II software at the University of Arkansas for Medical Sciences (FACScan; Becton Dickinson, Sunnyvale, Calif.). The machine was calibrated with beads (CaliBRITE; Becton Dickinson) by using AutoCOMP software. Dead cells were excluded on the basis of forward angle and 90° light scatter, and 10,000 cells were analyzed. The percentage of gamma-delta T-cell-positive cells was determined by gating with CD3-positive cells. IFN-γ levels in homogenized whole-lung material were determined by ELISA with monoclonal hybridomas XMG 1.2 and R4-6A2 as described previously (11). IL-10 levels in homogenized lung material were determined by using a kit (Genzyme, Cambridge, Mass.) according to the manufacturer's instructions. Antibodies to MoPn were detected by ELISA with a peroxidase-conjugated anti-mouse antibody as previously described (15). The antigen used for the ELISA was Renografin density-purified MoPn

elementary bodies (5 μg per well). For statistical analyses, Student's *t* test with correction for unequal variance and the Mann-Whitney U test were used.

Figure 1A shows the combined results of three experiments, each with 10 to 12 mice per group on days 3 and 7 and with 7 to 9 mice per group on day 20. B6129F2/J control mice and Tcrd^{tm1Mom} mice were infected with 10^4 viable MoPn elementary bodies on day 0, and the levels of the MoPn antigen in the lung were determined on days 3, 7, and 20 postinfection. A modest but significant elevation of MoPn levels was observed on both days 3 and 7 in the gamma-delta T-cell functionally deficient mice ($P < 0.05$ and $P < 0.03$, two-tail *t* test, on days 3 and 7, respectively). Because the B6129F2/J control is not an exact genetic match for the gamma-delta T-cell-deficient animals, separate experiments were performed on day 3 to compare the MoPn antigen levels in parental strain B129SvJ with those in the gamma-delta T-cell-deficient animals in groups of four or five animals each (Fig. 1B). The gamma-delta T-cell-deficient animals were again more susceptible ($P < 0.03$, two tail *t* test). Similarly, when C57BL/6 parental strain mice were compared with gamma-delta T-cell-deficient mice on day 3 (three or four mice per group), the latter were more susceptible ($P < 0.05$, one-tail *t* test) (Fig. 1C). Thus, a consistent increase in MoPn levels in the lungs was seen early in infection in the gamma-delta T-cell-deficient animals. The reason for the paradoxical effect at day 20 (Fig. 1A) in which an absence of gamma-delta T cells was apparently beneficial to host defense is not clear. In the first of the three experiments combined to produce the day-20 data (Fig. 1A), two animals with high MoPn counts in the B6129F2/J group died on day 19 and the data for these two mice were not included in the results shown. Despite not counting these animals, there was a consistent increase in MoPn levels in the gamma-delta T-cell +/+ animals compared with the −/− animals ($P < 0.05$, two-tail *t* test). To determine if this was an effect unique to this control group, a repeat experiment was run on day 20 with C57BL/6 controls. In this experiment with five mice per group, counts in the C57BL/6 controls tended to be higher ($P < 0.09$, one-tail *t* test) than those in the Tcrd^{tm1Mom} group, but the effect was less dramatic and a wider range of standard errors diminished the statistical significance. The mean counts ± standard errors of the means were (1,144 ± 342) × 10^4 IFU for the controls compared with (293 ± 88) × 10^4 IFU for the gamma-delta T-cell-deficient mice. Thus, although the degree of the paradoxically improved control of infection in the absence of gamma-delta T cells was less in C57BL/6 controls, a similar trend was seen. These data indicate that while the paradoxical effect may be exaggerated in the B6129F2/J control group, there appears to be a more general phenomenon that gamma-delta T-cell function can be deleterious, similar to that previously observed with salmonella infection and Chagas' disease (5, 9) (although in the case presented here, clearance of the organism is impaired in addition to an increase in mortality).

FACS of total lung cells was done on day 3 of infection to determine the percentages of gamma-delta T cells in B6129F2/J and gamma-delta T-cell-deficient mice by gating with $CD3^+$ cells. Percentages were 5.41 ± 1.84 and 0.26 ± 0.23, respectively ($P < 0.009$, two-tail *t* test), confirming that the Tcrd^{tm1Mom} mice are gamma-delta T-cell deficient. Similar data were obtained with whole spleen cells from the two groups (data not shown).

We next performed cytokine assays of lung tissue for IFN-γ and tumor necrosis factor alpha to determine if we could detect differences to explain the susceptibility patterns that we had observed. B6129F2/J control mice produced significantly more IFN-γ than Tcrd^{tm1Mom} mice on day 7 postinfection (510 ± 45 versus 340 ± 25 ng/ml, respectively; $P < 0.007$) but not on day 3 (data not shown), suggesting that the increase in MoPn levels observed at day 7 might be due in part to a deficiency of IFN-γ produced by gamma-delta T cells but not providing the confirmation of similar significant differences on day 3. Prior studies have shown a role for IFN-γ in host defense against MoPn in the lung in the model that we used (20). The measurement of tumor necrosis factor alpha (also shown to play a role in host defense in this model [21]) demonstrated no difference in the two mouse groups on any of the 3 days. Maximum production was 1,113 ± 248 pg/ml in B6129F2/J mice and 893 ± 252 pg/ml in Tcrd^{tm1Mom} mice on day 7 ($P = 0.23$), and the levels were 678 ± 117 and 588 ± 53 pg/ml in the two groups, respectively, on day 20 (*P*, not significant). Thus, the differences in mortality on day 20 could not be explained by a potentially deleterious increase in tumor necrosis factor alpha in the control animals compared with that in the gamma-delta T-cell-deficient mice, as was seen for salmonella infection (5). Since, in addition, IFN-γ levels tended to be lower, instead of higher, in the gamma-delta T-cell-deficient group, it was not clear that the increased mortality in controls was due to a deleteriously increased Th1 response. To determine if gamma-delta T cells might increase MoPn levels by overly downregulating the immune response on day 20, we measured IL-10 levels during that time period. No significant difference in levels of that cytokine for the two groups was observed. The levels in B129F2/J mouse lung tissues on day 20 were 141 ± 35 pg/ml compared with 150 ± 25 pg/ml in Tcrd^{tm1Mom} mice (*P*, not significant). In a separate experiment, no significant difference in the levels in lung tissues of C57BL/6 and Tcrd^{tm1Mom} mice was seen (data not shown). To the same end, the levels of antibody to MoPn in serum were also determined, but the relatively dehydrated state of the B129F2/J mice on day 20 prevented the collection of adequate amounts of serum from which to draw a reliable conclusion regarding differences from Tcrd^{tm1Mom} mice. Therefore, further study will be required to determine the mechanisms involved in the paradoxical reversal in susceptibility to MoPn observed on day 20.

These studies show that gamma-delta T cells play a modest positive role in host defense early in MoPn infection. Help in the production of IFN-γ might be one of the immunologic mechanisms involved, but this role has not been proven. The protective role appears to be substantially less, however, than that of alpha-beta T cells, which play a crucial role in host resistance in this infection (22). The apparent negative role that these cells play later in infection, possibly in part similar to the deleterious effects ascribed to an overly exuberant cellular immune response related to gamma-delta T cells in other infectious disease models (5, 9), and the possible relationship to autoimmunity and persistent HSP are the subjects of ongoing studies.

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