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Nematode Infection Enhances Survival of Activated T Cells by Modulating Accessory Cell Function

Robert S. Liwski*† and Timothy D. G. Lee2*†

The type of immune response generated following exposure to Ag depends on a variety of factors, including the nature of the Ag, the type of adjuvant used, the site of antigenic entry, and the immune status of the host. We have previously shown that infection of rodents with *Nippostrongylus brasiliensis* (*Nb*) shifts the development of type 1 allo-specific responses toward type 2 immunity, suggesting nematode modulation of T cell activation. In this report we explore the immunomodulatory effects of *Nb* on T cell activation. We found that spleen cells from *Nb*-infected mice exhibited dramatically increased proliferation in response to Con A and anti-CD3. This hyperproliferation could be transferred in vitro to naive splenocytes by coculture with mitomycin C-treated cells from *Nb*-infected animals. The transfer was mediated by non-T accessory cells and supernatants derived from Con A-activated non-T cells, suggesting the involvement of a soluble factor secreted by accessory cells. The accessory cells secreted high levels of IL-6, and anti-IL-6 treatment abrogated the supernatant-induced hyperproliferation, thus confirming that IL-6 was mediating the effect. Further, spleen cells from *Nb*-infected mice were more resistant to activation-induced cell death (AICD) following mitogenic stimulation. Reduced AICD was also transferable and IL-6 dependent. Thus, the hyperproliferation was in part due to enhanced activated T cell survival. These phenomena mediated by accessory cells may contribute to the powerful polyclonal activation of type 2 immunity caused by nematode infection. The Journal of Immunology, 1999, 163: 5005–5012.

Infection with the nematode parasite *Nippostrongylus brasiliensis* (*Nb*) induces strong type 2 immune responses in mice and rats (reviewed in Refs. 1–3), which include mastocytosis (4, 5), eosinophilia (6), and a dramatic increase in the production of IgE (7, 8). These responses are similar to those noted in many nematode infections in humans and animals (9–11). As such, *Nb* has been used by many researchers as a model for immune effects mediated by nematode infection. These potent responses have been suggested to result from a marked activation of IL-4-producing T lymphocytes (4, 6, 7). *Nb* infection induces a strong proliferative T cell response in vivo (12–14) and exhibits stable TCR Vβ-chain usage (13, 14). These data taken together with the fact that most of the IgE induced by *Nb* is not specific to the worm (12, 15) suggest polyclonal T cell activation. Indeed, *Nb* infection results in a 4-fold increase in the frequency of IL-4-secreting CD4+ T cells (16), confirming the polyclonal nature of the immune hyperactivation of the T cell compartment of the immune responses during *Nb* infection. However, the mechanisms underlying this activation have not previously been described.

The development of type 2 immunity during nematode infection is dependent on both the cytokine milieu (17, 18) and the type of costimulatory signals provided by the APC (19) during activation. The presence of IL-4 is necessary for the induction of Th2 responses associated with *Nb* infection, because IL-4-deficient (20, 21) or STAT6-deficient (22) mice do not exhibit blood eosinophilia (20) and do not produce IgE (20–22) or type 2 cytokines (20, 22) in response to *Nb* infection. In addition to IL-4, recent evidence indicates that IL-6 is also an important factor for the induction of Th2 cells (23–25). Rincon et al. (23), for example, have shown that IL-6, secreted from APC, stimulates IL-4 production from T cells in response to polyclonal T cell activators as well as specific peptide Ags. Interestingly, alveolar macrophages produce high levels of IL-6 (26, 27) in the very early stages (starting from day 1) of *Nb* infection, suggesting a role for these APC in the early development of type 2 T cells.

Previous studies in our laboratory have demonstrated that infection with *Nb* prolongs allograft survival in rats (28) and mice (29). This was associated with immunomodulatory effects by *Nb* on developing allo-specific T cell responses induced by immunization with allogeneic cells. These effects included a depression of allo-specific CTL activity, a marked inhibition of IFN-γ production, and a dramatic increase in secretion of IL-4 and IL-6 (29). Interestingly, spleen cells from *Nb*-infected mice, not previously immunized with alloantigen, also elaborated greater than control levels of IL-4 and very high levels of IL-6 upon primary activation with allogeneic cells, further confirming that polyclonal type 2 activation occurs. Taken together these results suggested that infection with *Nb* had a dramatic effect on the development of immune responses to unrelated Ags and that these effects may be mediated through IL-6.

In this study we investigated the effects of *Nb* on in vitro T cell activation in response to polyclonal T cell activators. We found that spleen cells from *Nb*-infected mice exhibited proliferative hyper-responsiveness upon activation with Con A and anti-CD3. This effect could by transferred in vitro with accessory non-T cells from *Nb*-infected mice and was found to be mediated by IL-6 produced by these cells. In addition, this hyper-responsiveness was in large part due to induction of resistance to AICD. The increased T cell proliferation and reduced AICD that occur in the type 2 cytokine environment induced by *Nb* may explain how exaggerated type 2 T cell responses are generated and persist during nematode
infection. The elaboration of this and other immunomodulatory effects of nematodes is of great interest, because a substantial part of the world’s population encounters Ag, both naturally and in the form of vaccines, against a background of nematode infection (10).

Materials and Methods

Animals

Male 6- to 8-week-old C57BL/6J (H-2b) and BALB/cByJ (H-2d) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in the Medical Sciences animal care facility with food and water ad libitum for 2 wk before experimentation.

Nippostrongylus brasiliensis

The life cycle of Nb was maintained in Sprague Dawley rats (Harlan, Indianapolis, IN) as we have previously described (28). Eight hundred third-stage larvae of Nb in 200 µl of PBS were used for inoculation of mice.

In vivo treatment

Groups of 8- to 12-week-old male C57BL/6 mice were infected s.c. with Nb. Control mice were not treated. On day 11 after infection all mice were sacrificed by cervical dislocation, and spleen cells were isolated for in vitro culture.

Cell isolation

Single-cell suspensions from spleens of C57BL/6 mice were prepared as described previously (29, 30) under aseptic conditions in RPMI (ICN, Aurora, OH) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (Life Technologies, Burlington, Canada), 10% FBS (Life Technologies), 20 mM HEPES (Life Technologies), and 50 µM 2-ME (Sigma, St. Louis, MO). Cells were pooled within experimental groups. Erythrocytes were lyzed by lysis with ammonium chloride/potassium bicarbonate lysing buffer for 2 min at room temperature. Cells were washed three times and resuspended in RPMI for in vitro culture. In coculture experiments some cell populations were inactivated with 25 µg/ml of mitomycin C (Sigma) for 30 min at 37°C. In separate cultures, these mitomycin C-treated cells exhibited 88–94% inhibition of proliferation in response to Con A. In some coculture experiments T cells were depleted from the spleen cell population by treatment with anti-Thy1.2 mAb (mouse IgG2b; PharMingen) were added at the beginning of culture. To block conditioned supernatant or medium alone (final vol-

permanant- or IL-6-mediated enhancement of spleen cell proliferation, varied concentrations (ranging from 10–50 ng/ml) of anti-IL-6 mAb (rat IgG1 anti-mouse; clone MP5-20F3; PharMingen) were added to wells containing supernatant or mouse IL-6, and the plates were incubated at 37°C for 1 h before addition of spleen cells and initiation of the proliferation assay.

Detection of AICD by JAM assay

AICD was measured by using the JAM assay (31). Briefly, spleen cells (2 × 10^5/well) from Nb-infected or naive mice were activated with Con A (5 µg/ml) in two sets of replicate wells per group in 96-well round-bottomed plates. After 24-h incubation, one set of six replicates (pulsed set) was pulsed with 1 µCi/well of [ ^3 ]H]TdR, while the second set of six replicates was left untreated (untreated set). Eighteen hours later the contents of the wells within each set were collected into centrifuge tubes. Cells from the pulsed sets were washed twice with RPMI to prevent further incorporation of [ ^3 H]TdR into the cells and resuspended in the supernatant from the corresponding untreated set to preserve the culture conditions during subsequent culture. The cells were then replated in two sets of triplicates. The first set was harvested (cell harvester) immediately (T_0h), whereas the second set was harvested after 24-h incubation (T_24h) at 37°C. The loss of radiolabeled DNA during 24-h incubation was used to measure the extent of DNA fragmentation in the cells that proliferated during the pulsing period. Specific AICD was calculated using the following formula: % specific AICD = (T_0h − [ ^3 ]H]TdR in cells at T_0h)/(T_0h − [ ^3 ]H]TdR in cells at T_24h) × 100%. Resistance to AICD was calculated using the following formula: % resistance = (% AICD of experimental treatment group/% AICD of naive control group) × 100%. In coculture experiments 2 × 10^5 responder cells were used in the presence of 1 × 10^5, mitomycin C-treated stimulator cells. To assess the effects of conditioned supernatant or IL-6 on AICD, the supernatant or the cytokine (at various concentrations) was added at the beginning of primary cultures in the proliferation assay. To block conditioned supernatant- or IL-6-mediated resistance to AICD, anti-IL-6 mAb (final concentration, 50 ng/ml) was added to the wells containing various supernatants or mouse IL-6, and the plates were incubated at 37°C for 1 h before addition of spleen cells and initiation of proliferation assay.

ELISA for detection of cytokines

Supernatants were analyzed for presence of IFN-γ, IL-4, IL-6, and IL-10 by a sandwich ELISA. All mAb and mouse recombinant cytokine standards used were purchased from PharMingen. The capture Abs used were rat IgG1 anti-mouse; clone YTS 169.4; rat IgG2a anti-mouse; clone 2B11; rat IgG1 anti-mouse; clone MP5-20F3, and anti-IFN-γ (rat IgG1; clone R4-6A2); the biotinylated Abs used were anti-IL-4 (rat IgG1 anti-mouse; clone XMG1.2). Briefly, ELISA plates (Costar, Cambridge, MA) were coated with anti-cytokine mAb in carbonate buffer (pH 9.0) at 4°C overnight. After overnight incubation and blocking with 2 mg/ml BSA (Life Technologies) in Tris-buffered saline, test samples were added in a 10-fold serial dilution (starting at 10 µg/ml) in the presence of mouse recombinant cytokines and supernatants were added. The plates were incubated and incubated overnight at 4°C. Cytokines were detected using biotinylated anti-cytokine mAb, Extravidin-peroxidase (Sigma), and tetramethylbenzidine substrate solution (Life Technologies). Detection limits were 50 pg/ml for IFN-γ and 15 pg/ml for IL-4 and IL-6.

FACS analysis

Cells (1 × 10^6) were washed twice in PBS containing 1% BSA, then incubated in the dark with 2 µg/ml anti-CD4 mAb (rat IgG2b anti-mouse; clone YTS 191.1; Cedarlane), anti-CD8α mAb (rat IgG2b anti-mouse; clone YTS 169.4; Cedarlane), or isotype control (rat IgG2b; Cedarlane) at 4°C for 30 min. Cells were then washed three times, fixed in PBS containing 0.1% paraformaldehyde, and stored at 4°C overnight. Flow cytometric analysis was performed on a FACScan (Becton Dickinson) using LYSIS II software.

Results

Spleen cells from Nb-infected mice exhibit enhanced responsiveness to Con A and anti-CD3 Ab

We have previously found that infection of mice with Nb inhibited allo-Ag-specific CTL activity if the immunization with allo-Ag took place 4 days after Nb infection (29). To confirm that Nb did not induce a nonspecific suppression of T cell responsiveness, we compared mitogen-induced proliferative activity of splenic T cells from Nb-infected and uninfected mice. Spleen cells from

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FIGURE 1. Infection of mice with Nb results in enhanced spleen cell proliferation in response to T cell mitogens. Proliferation of spleen cells from uninfected and Nb-infected mice in response to Con A (A) or anti-CD3 mAb (B) was assessed by [3H]Tdr incorporation. Splenocytes were isolated from mice 11 days after infection and stimulated with Con A (5 μg/ml) or anti-CD3 mAb (1/20 hybridoma supernatant) for 72 h before pulsing with [3H]Tdr. Data shown in A are expressed as the mean ± SEM of six separate experiments (***, p < 0.001, by t test). Data shown in B are expressed as the mean ± SD of triplicate wells and are representative of four experiments (***, p < 0.001, by t test).

Nb-infected C57BL/6 mice exhibited significantly (p < 0.001) enhanced proliferation in response to both Con A (Fig. 1A) and anti-CD3 (Fig. 1B) compared with cells from control uninfected mice. Similar results were obtained when BALB/c mice were used instead of C57BL/6 mice (129% increase over control for Con A stimulation; data not shown), confirming that Nb-induced hyperresponsiveness to the T cell mitogen is not a strain-specific phenomenon. There was no difference in the background proliferation exhibited by spleen cells isolated from infected or uninfected mice (data not shown). The increased proliferative response seen with spleen cells from Nb-infected mice did not result from an increase in the proportion of T cells in the spleen, because the percentages of CD4+ and CD8+ T cells in the spleens from Nb-infected mice (assessed by flow cytometry) were not different from those observed in uninfected controls (uninfected CD4+, 15.63 ± 2.28; Nb-infected CD4+, 14.08 ± 1.98; uninfected CD8+, 15.03 ± 2.66; Nb-infected CD8+, 15.23 ± 1.13; n = 3).

Non-T spleen cells from Nb-infected mice transfer increased responsiveness to naive spleen cells in vitro

To investigate the mechanism by which Nb-induced hyperresponsiveness to Con A is manifest, we assessed whether cells from Nb-infected mice could transfer the effect to naive spleen cells in vitro. Naive splenocytes were stimulated in vitro with Con A in the presence of spleen cells that had been isolated from Nb-infected animals and inactivated with mitomycin C (transferring cells).

Non-T spleen cells from Nb-infected mice can transfer increased proliferation to naive spleen cells. Proliferation of spleen cells from Nb-infected mice significantly (p < 0.001) enhanced proliferation to Con A to naive spleen cells was assessed by coculture experiments. Naive spleen cells were activated with Con A in the absence of any transferring cells (none) or in the presence of mitomycin C-inactivated transferring cells from either Nb-infected (Nb) or uninfected (uninfected) mice. Mitomycin C-inactivated transferring cells were either spleen cells (A) or T cell-depleted spleen cells (B). All data are expressed as a percentage of the proliferative response in naive (control) spleen cells (i.e., in the absence of transferring cells). Data shown in A are expressed as the mean ± SEM of nine separate experiments (***, p < 0.001, by repeated measures ANOVA). Data shown in B are expressed as the mean ± SEM of 12 separate experiments (***, p < 0.001, by repeated measures ANOVA).

Similarly treated transferring cells from uninfected mice were used as controls. Fig. 2A shows that the addition of mitomycin C-inactivated transferring spleen cells from Nb-infected mice significantly (p < 0.001) enhanced Con A-induced proliferation of naive spleen cells (by ~260%). If the transferring cells were taken from uninfected mice, rather than Nb-infected mice, the proliferation was increased only mildly. These data indicate that the Nb-induced increase in proliferation can be transferred by coculture to naive cells even if the transferring cells are mitomycin C-treated. Similar results were obtained in BALB/cByJ mice (data not shown).

Because accessory cell costimulation is necessary for the induction of T cell proliferation to Con A (32, 33), the observed changes in proliferation could be due to modification of either T cell or accessory cell function. To address this question we examined whether a mitomycin C-inactivated, T cell-depleted, spleen cell population from Nb-infected mice could transfer the observed hyperresponsiveness onto naive spleen cells. Spleen cells were depleted of T cells by treatment with anti-Thy1.2 Ab and complement. The efficiency of T cell depletion was tested by inhibition of responsiveness to Con A as well as by flow cytometry and was always >98% (data not shown). When added as coculture cells, mitomycin-C-inactivated, T cell-depleted, transferring spleen cells from Nb-infected mice significantly
augmented Con A-induced proliferation of naive syngeneic cells (by $\sim 180\%$; Fig. 2B). Similar cells from uninfected animals did not show this dramatic effect (Fig. 2B).

**Hyper responsiveness to Con A induced by spleen cells from Nb-infected mice is mediated by a soluble factor**

Accessory cells can enhance proliferative T cell responses through expression of membrane-bound costimulatory molecules such as B7 (32, 34) or ICAM-1 (34, 35, 36) or through secretion of soluble factors, including IL-1 (37, 38), TNF-$\alpha$ (37), and IL-6 (37–39). To ascertain whether a soluble factor (or factors) was responsible for the induction of hyper-responsiveness, we collected 24-h supernatants from Con A-stimulated cultures of spleen cells from either Nb-infected or naive mice. We tested these supernatants for their ability to enhance Con A-induced proliferation of naive spleen cells. Fig. 3 shows that supernatants from Con A-stimulated spleen cells from uninfected mice do not significantly enhance the proliferation of naive spleen cells in response to Con A. In contrast, supernatants of Con A-stimulated spleen cells from Nb-infected mice markedly enhanced Con A-induced proliferation of naive spleen cells. This suggested that spleen cells from Nb-infected mice elaborated a factor(s), in response to Con A that enhanced proliferation. To investigate whether non-T cells were the source of this stimulatory factor, we generated 24-h supernatants from Con A-stimulated cultures of spleen cells from either Nb-infected or naive mice. We tested these supernatants for their ability to enhance Con A-induced proliferation of naive spleen cells. Fig. 3 shows that supernatants from Con A-stimulated spleen cells from uninfected mice do not significantly enhance the proliferation of naive spleen cells in response to Con A. In contrast, supernatants of Con A-stimulated spleen cells from Nb-infected mice markedly enhanced Con A-induced proliferation of naive spleen cells. This suggested that spleen cells from Nb-infected mice elaborated a factor(s), in response to Con A that enhanced proliferation. To investigate whether non-T cells were the source of this stimulatory factor, we generated supernatants from cultures of Con A-activated non-T cells and assessed their ability to enhance proliferation of naive spleen cells in response to Con A. Fig. 3 demonstrates that supernatants from stimulated non-T cells from Nb-infected mice effectively induced hyper-responsiveness to Con A.

To investigate the nature of the factor mediating this effect we analyzed the supernatants for cytokine content by ELISA. We found that levels of production of IFN-$\gamma$ (Fig. 4A) were decreased in the cultures of Con A-stimulated spleen cells from Nb-infected mice. However, these same cultures showed a dramatic up-regulation of type 2 cytokine production, including IL-4 (Fig. 4B), IL-6 (Fig. 4C), and IL-10 (data not shown). This was not surprising, because Nb infection is known to result in polyclonal activation of type 2 T cells (13, 14, 16). T cell depletion resulted in complete inhibition of IFN-$\gamma$ (Fig. 4A) and IL-10 (data not shown) production, suggesting that secretion of these cytokines in response to Con A is either mediated by or dependent on T cells. Secretion of IL-4 in response to Con A was T cell dependent to a large extent, because T cell depletion resulted in $\sim 83\%$ inhibition of IL-4 production (Fig. 4B). In contrast, high levels of IL-6 were secreted by Con A-stimulated spleen cells from Nb-infected mice even when T cells were depleted (Fig. 4C). This confirms that at least 50% of the IL-6 production in response to Con A was accessory cell dependent.

To determine whether any of these cytokines could represent the factor responsible for the hyper-responsiveness seen above, we tested recombinant murine IL-4, IL-6, and IL-10, at concentrations approximating those found in the supernatants, for their ability to mimic the effects of the transferring supernatant. Fig. 5 confirms that IL-6, at the concentrations found in the stimulated culture supernatants of T cell-depleted spleen cells from Nb-infected mice, significantly enhanced the proliferation of naive spleen cells in

**FIGURE 3.** Supernatants of spleen cells or T cell-depleted spleen cells from Nb-infected mice enhance proliferation of naive spleen cells to Con A. Naive spleen cells were activated with Con A in the presence of supernatants derived from Con A-activated spleen cells from Nb-infected (Nb) or uninfected mice. Naive spleen cells were also activated in the presence of supernatants derived from Con A-activated, T cell-depleted spleen cells (uninfected non-T, Nb non-T). All supernatants were tested at a final culture concentration of 1/4. All data are expressed as a percentage of proliferation exhibited by naive spleen cells in the absence of any supernatant. Data are expressed as the mean $\pm$ SEM of five (for whole spleen cell supernatants) and nine (for non-T cell supernatants) separate experiments ($***$, $p < 0.001$, by repeated measures ANOVA).

**FIGURE 4.** Con A-stimulated spleen cells from Nb-infected mice produce enhanced levels of IL-4 and IL-6 but reduced levels of IFN-$\gamma$. Production of cytokines by spleen cells or mitomycin C-treated T cell-depleted spleen cells from uninfected or Nb-infected mice was assessed by ELISA. Cells were activated with Con A for 24 h. Supernatants were analyzed for the presence of IFN-$\gamma$ (A), IL-4 (B), and IL-6 (C). Data shown are expressed as the mean $\pm$ SEM of four (IFN-$\gamma$) or nine (IL-4 and IL-6) experiments ($***$, $p < 0.001$, by repeated measures ANOVA). ND, not detectable.
response to Con A. In contrast, recombinant mouse IL-4 and IL-10 (data not shown) had no effect on Con A-induced proliferation. This strongly suggested that IL-6 was the accessory cell factor responsible for transferring the hyper-responsiveness to naive cells. To confirm this, we inhibited this effect by treatment of the transferring supernatant with anti-IL-6 Ab (Fig. 6). When activated supernatants from uninfected mice were used, no enhanced proliferation was seen (as above), and this was not significantly affected by anti-IL-6 treatment, thus confirming that the mAb had no nonspecific inhibitory effects (Fig. 6). Moreover, as an additional control we confirmed that anti-IL-6 treatment completely abrogated the rIL-6-mediated enhancement of spleen cell proliferation in response to Con A, as demonstrated above, and that this blocking effect of Ab could be abolished by adding high levels of IL-6 (5 ng/ml) to the cultures (data not shown). Taken together these results confirm that IL-6, present in the supernatant, is the factor responsible for induction of proliferative hyper-responsiveness to Con A.

**Infection with Nb results in decreased susceptibility of Con A-activated spleen T cells to AICD**

Nb-induced proliferative hyper-responsiveness may be due either to an increase in the number of cells that are stimulated to enter the cell cycle or to a reduction in the rate of AICD of cells that have progressed through the cell cycle. A kinetic study of the Con A-induced proliferation demonstrated that naive spleen cells attain nearly maximal proliferation at 42 h. From that time on proliferation levels plateau, exhibiting only a marginal increase over the next 48 h (Fig. 7A). In contrast, spleen T cells from Nb-infected mice exhibited a dramatic increase in the rate of proliferation at each time point assessed, starting from 22 h until 90 h. Based on those results and the fact that IL-6 has been shown to rescue T cells from undergoing apoptosis (39–41), we hypothesized that infection with Nb may lead to a decrease in susceptibility to AICD.

To test this hypothesis we activated spleen cells from control or Nb-infected mice with Con A and performed the JAM assay to confirm that IL-6, present in the supernatant, is the factor responsible for induction of proliferative hyper-responsiveness to Con A.

**FIGURE 5.** Mouse rIL-6 enhances spleen cell proliferation in response to Con A. Naive spleen cells were activated with Con A in the absence or the presence of mouse rIL-6 at varying concentrations. All data are expressed as a percentage of the proliferation exhibited by naive spleen cells in the absence of rIL-6. Data are expressed as the mean ± SEM of three (for 0.31 ng/ml of IL-6) or eight (all other groups) separate experiments (***, p < 0.001, by one-way ANOVA). *A total of 1.25 ng/ml is the average concentration of IL-6 in the test proliferation cultures containing 1:4 Nb non-T supernatant.

**FIGURE 6.** Anti-IL-6 treatment abrogates supernatant-induced hyper-responsiveness to Con A. Naive spleen cells were activated with Con A in the presence of a supernatant derived from Con A-stimulated T cell-depleted spleen cell cultures from Nb-infected (Nb non-T) or uninfected (uninfected non-T) mice (1/4 final concentration). Cultures received 10 ng/ml ( [], 25 ng/ml ( [ ) or 50 ng/ml ( [ ) anti-IL-6 Ab or no Ab ( [ ]). Data shown are expressed as the mean ± SEM of three separate experiments (***, p < 0.001; NS, not significant; by repeated measures ANOVA).

**FIGURE 7.** Increased proliferation induced by Nb infection is associated with resistance to AICD, which can be transferred with non-T cells in vitro. In A Con A-induced proliferation of spleen cells from uninfected (■) and Nb-infected ( [] mice was assessed by [3H]Tdr incorporation at various time points. [3H]Tdr was added 18 h before termination of cultures. Data shown are expressed as the mean ± SEM of three separate experiments (**, p < 0.01; ***, p < 0.001; NS, not significant; by repeated measures ANOVA). In B the extent of AICD was assessed in spleen cell cultures from naive and Nb-infected mice as well as in cocultures of naive spleen cells with non-T spleen cells from either Nb-infected or uninfected mice. AICD was assessed using the JAM test, and the percent resistance in AICD relative to the AICD exhibited by naive spleen cells was determined as described in Materials and Methods. Data are expressed as the mean ± SEM of five or six separate experiments (***, p < 0.001, by one-way ANOVA). *ND, not different from AICD exhibited by naive cells in the absence of any transferring cells.
their effect, we found that recombinant mouse IL-6 at levels matching those present in the supernatants was also able to induce resistance to AICD (Fig. 8A). Further, anti-IL-6 treatment abrogated both the supernatant-induced (Fig. 8B) and rIL-6-induced (from 39.8% to 5.0%; data not shown) resistance to AICD. These results suggest that Nb-induced hyper-responsiveness to Con A can be in large part attributed to decreased AICD after stimulation.

Discussion

Nematode infection induces a marked type 2 T cell response (1–3), and considerable evidence points to this response being polyclonal in nature (12–16). Previously, we have shown that nematode infection (28, 29) as well as extracts (42) exacerbate type 2 responses to unrelated Ags, suggesting that nematode infection can modify the development of immune responses to infection or vaccination. The manner in which this immune deviation is mediated is unclear. The results of this study show that spleen cells from Nb-infected mice exhibit dramatic hyperproliferation upon in vitro stimulation with the T cell activators Con A and anti-CD3 mAb. This modification of T cell responsiveness could be transferred to naive spleen cells in vitro by coincubation with spleen cells from Nb-infected mice. Interestingly, non-T accessory cells from Nb-infected mice were sufficient to transfer this effect, suggesting that Nb infection induced an enhancement of costimulatory function in the accessory cell population.

In addition to this, we demonstrated that a soluble factor present in the supernatant of stimulated non-T cells from Nb-infected mice could confer this hyper-responsiveness on naive T cells when added to naive T cell cultures along with Con A. Given the evidence in the literature regarding the enhancing effects of IL-6 on T cell proliferation (37–39) and our data, we hypothesized that the soluble factor could be IL-6. We found, for example, that IL-6 was secreted in response to Con A by the non-T cells from Nb-infected mice. In addition, mouse rIL-6, at a concentration matching that found in the transferring supernatant, increased Con A-induced naive spleen cell proliferation to the same extent as the supernatant. We tested this hypothesis using an mAb to IL-6 to block IL-6 activity in the supernatant. Anti-IL-6 mAb treatment completely abrogated the supernatant-induced hyper-responsiveness, confirming that IL-6 was responsible for the effect. This anti-IL-6 mAb had an identical effect on the hyper-responsiveness induced by rIL-6. The efficiency of transfer of hyperproliferation to naive spleen cells was very similar for coculture with non-T cells from Nb-infected mice (100% increase compared with that in non-T cells from uninfected mice), non-T cell supernatants (92% increase compared with that in non-T cells from uninfected mice), and rIL-6 (100% increase compared with medium control value), suggesting that IL-6 elaboration by these accessory cells is the sole cause of this effect.

It is unclear at this point whether IL-6 mediates its effects by direct action on T cells or indirectly through accessory cells. Our recent experimentation (R. S. Liwski and T. D. G. Lee, unpublished observations) has revealed that B7.2 is overexpressed by some cases lead to increased proliferative T cell activity (32, 34). Whether induction of B7.2 expression was mediated through IL-6 in the supernatant of stimulated non-T cells from Nb-infected mice did not have this effect (Fig. 7B). Because the hyperproliferation could be transferred with supernatants, we also assessed whether supernatants from Con A-stimulated spleen cells from Nb-infected mice would likewise reduce AICD. We found that supernatants from Nb-infected mice did confer resistance to AICD on naive spleen cells upon stimulation with Con A (Fig. 8A). In support of our hypothesis that IL-6 in these supernatants is responsible for

assess apoptosis as a measure of AICD. As shown in Fig. 7B spleen cells from Nb-infected mice exhibited a significant (46%; n = 10; p < 0.001) resistance to AICD compared with cells from uninfected mice. Further, coculture of naive spleen cells with mitomycin C-treated, non-T-spleen cells from Nb-infected mice resulted in a significant increase in resistance to AICD after stimulation with Con A. Coculture of naive spleen cells with non-T cells from uninfected mice did not have this effect (Fig. 7B). Because the hyperproliferation could be transferred with supernatants, we also assessed whether supernatants from Con A-stimulated spleen cells from Nb-infected mice would likewise reduce AICD. We found that supernatants from Nb-infected mice did confer resistance to AICD on naive spleen cells upon stimulation with Con A (Fig. 8A). In support of our hypothesis that IL-6 in these supernatants is responsible for

FIGURE 8. Nb-induced resistance to apoptosis can be transferred with non-T cell supernatants and is mediated by IL-6. In A the extent of AICD was assessed in spleen cell cultures from naive mice stimulated in the absence or the presence of supernatants derived from cultures of Con A-activated non-T cell from Nb-infected (Nb non-T sup) or uninfected (uninfected non-T sup) mice or rIL-6. Data shown are expressed as the mean ± SEM of three separate experiments (***, p < 0.001, by repeated measures ANOVA). In B AICD was assessed on naive spleen cells that were activated with Con A in the absence or the presence of a supernatant derived from Con A-activated T cell-depleted spleen cell cultures from Nb-infected (Nb non-T) or uninfected (uninfected non-T) mice (1/4 final concentration). In addition, cultures received 50 ng/ml anti-IL-6 Ab (■) or no Ab (□). Data shown are expressed as the mean ± SEM of three separate experiments (***, p < 0.001; NS, not significant; by repeated measures ANOVA). In each case AICD was assessed using the JAM test, and the percent resistance in AICD relative to the AICD exhibited by naive spleen cells was determined as described in Materials and Methods. *A total of 1.25 ng/ml is the average concentration of IL-6 in the test proliferation cultures containing 1:4 Nb non-T supernatant.
44), possibly by induction of apoptosis through up-regulation of Fas ligand expression (45). Further, IFN-γ production from mitogen-activated T cells can be dramatically reduced by IL-6 (23). Our data clearly showed that spleen cells from Nb-infected mice produced significantly lower levels of IFN-γ in response to Con A. However, addition of high levels of rIFN-γ did not diminish proliferation of naive spleen cells in response to Con A, blockage of IFN-γ activity by Ab treatment did not result in hyper-responsiveness, and coinoculation of naive spleen cells with non-T cells from Nb-infected mice did not decrease IFN-γ production in response to Con A (R. S. Liwski and T. D. G. Lee, unpublished observations). Therefore, it is unlikely that IL-6 acted through down-regulation of IFN-γ production to mediate the hyper-responsiveness observed in our studies.

The type of accessory cell that is responsible for Nb-mediated hyper-responsiveness to Con A is unclear. Both macrophages (46, 47) and B cells (48) can become activated with Con A and contribute to hyper-responsiveness. Moreover, Nb infection leads to the appearance of an IgE receptor bearing non-B, non-T cell population in the spleen (49), which may also be able to become activated with Con A. Mitogen activation of these non-T cells is not required to induce hyper-responsiveness, because spleen cells from Nb-infected mice show hyper-responsiveness to activation with anti-CD3. Moreover, non-T cells from Nb-infected mice induce increased proliferation when used as stimulators in an allogeneic mixed lymphocyte reaction (R. S. Liwski and T. D. G. Lee, unpublished observations).

Non-T accessory cells from Nb-infected mice were slightly less effective as transferring cells than whole spleen cells at enhancing of the proliferative response of naive cells. Thus, T cells from Nb-infected mice may be producing another factor, independent of accessory cells, that further enhances the IL-6 effect. For example, although the levels of IL-4 present in the supernatants from either whole spleen cells or non-T cells derived from Nb-infected animals were too low to induce hyper-responsiveness, the presence of IL-4–producing cells in the coculture may create high local concentrations of IL-4 and subsequently slightly increase the IL-6–mediated hyperproliferation.

Our data suggest that the nematode reduces AICD in mitogen-activated cells. A kinetic study of the Con A–induced proliferation demonstrated that naive spleen cells attain nearly maximal proliferation at 42 h. From that time on thymidine uptake plateaus, exhibiting only a marginal increase over the next 48 h. If all the cells that have gone through the cell cycle were capable of entering another cycle, we would expect a constantly higher levels of proliferation at later time points. Because thymidine uptake by naive spleen cells was relatively constant, it suggests that a large proportion of the dividing T cells in the naive spleen cell population do not re-enter the cell cycle or are unable to complete the cycle. In contrast, spleen T cells from Nb-infected mice exhibited a dramatic increase in the rate of proliferation at each time point assessed, starting from 22 h until 90 h. This progressive increase in thymidine uptake suggests that the majority of cells in spleen cell cultures from Nb-infected mice re-enter the cell cycle. Based on those results we hypothesized that infection with Nb leads to a decrease in susceptibility to AICD.

Assuming that all the cells that did not re-enter the cell cycle had died due to AICD, the estimated rate of AICD (based on data in Fig. 7a) between the 42 and 66 h points (assuming a 24-h cell cycle) was 56% for naive spleen cells and 25% for spleen cells from Nb-infected mice. Experimental assessment of AICD performed by the JAM assay showed that while 60.2% of naive spleen cells underwent AICD, only 35.1% of spleen cells from Nb-infected mice died in that same 24-h period (46% reduction of AICD). These data were very similar to the estimation of AICD derived from the data presented in Fig. 7a, suggesting that enhanced proliferation could be explained by reduction in AICD. Furthermore, the enhanced survival could be transferred in the same manner as the transfer of hyper-responsiveness as judged by proliferation. In addition, resistance to AICD conferred by supernatants could be blocked by anti-IL-6 treatment. Taken together these results confirm that the hyper-responsiveness induced by Nb is due to IL-6 mediation of resistance to AICD.

Our results are in agreement with the studies of Takeda et al. (39), who showed that IL-6 increased the proliferation of T cells in response to both Con A and anti-CD3 by inhibiting AICD through a Bcl-2–independent mechanism. It has been suggested that IL-6–induced inhibition of AICD may be mediated by down-regulation of Fas and Fas ligand expression on T cells (50). Because Fas ligand-mediated apoptosis plays a crucial role in the regulation of the survival of activated T cells in vivo (reviewed in Refs. 51 and 52), this link with IL-6 indicates that modulation of local IL-6 levels by nematodes could have profound effects on developing immune responses through mediating enhanced survival of activated T cells.

In this context, it is of interest that superantigen-activated T cells can be rescued from undergoing apoptosis by injection of LPS (53, 54). This has been shown to be mediated through up-regulation of proinflammatory cytokines (53, 54). Similar effects were observed during infection with vaccinia virus (55). These results suggest that inflammatory reactions induced by microbial infection profoundly modulate the development of other T cell–mediated responses. Interestingly, Rocken et al. (13, 14) demonstrated that infection with Nb also reactivates superantigen–specific T cell in mice previously tolerized with superantigen and induces type 2 T cell responses toward the superantigen.

A recent report by Zhang et al. (56) showed that T cells activated in vitro in a type 2 polarizing environment were highly resistant to AICD. Similar resistance to AICD was observed in Th2, but not Th1, clones (57). Others (58) have suggested that this preferential susceptibility of Th1 cells to undergo AICD may explain the development of nonprotective type 2 responses during HIV infection. Interestingly, Ranger et al. demonstrated that mice lacking both NF-ATp and NF-AT4 exhibit a dramatic increase in type 2 responses (59). This has been shown to be associated with hyperproliferation and resistance to AICD of T cells stimulated with anti-CD3 Abs (59), thus suggesting that uncontrolled overproduction of type 2 cytokines can lead to similar dramatic alterations in T cell responsiveness, as we have seen with Nb infection.

Our studies demonstrate that in vivo activation of type 2 T cells during Nb infection results in resistance to AICD. We have identified the factor, IL-6, that we believe is responsible for this enhanced T cell survival and subsequent hyperproliferation. Our studies suggest that modulation of IL-6 levels due to concurrent nematode infection could dramatically alter T cell–mediated responses to infections and/or vaccination in endemic areas.

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References


