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Protective Responses against Skin-Dwelling Microfilariae of *Onchocerca lienalis* in Severe Combined Immunodeficient Mice

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Inoculation of severe combined immunodeficient (SCID) mice with microfilariae of *Onchocerca lienalis* results in a sustained infection of the skin, extending for months beyond the point at which the parasites are eliminated from immunocompetent BALB/c controls. Reconstitution of SCID mice with spleen cells, thymocytes, or $CD4^+$-cell-enriched splenocytes from naive BALB/c donors confers the ability to mount a protective immune response, leading to the rapid elimination of microfilariae. High levels of interleukin-5 and low levels of gamma interferon in the sera of reconstituted SCID mice during the destruction of microfilariae suggest that this protective immune response is directed by Th2 lymphocytes, mirroring that observed in immunocompetent controls. Unexpectedly, abbreviation of primary infections of unreconstituted SCID mice with the drug ivermectin induces resistance to reinfection with microfilariae at a level equivalent to that induced in secondarily infected, immunocompetent controls. In contrast to protection mediated by adoptive reconstitution, resistance induced by ivermectin-abbreviated infection occurs in the absence of T cells and in association with negligible levels of serum interleukin-5 and gamma interferon. This points to the activation of some alternative host defense mechanism that operates after the clearance of therapeutic levels of drug. Such a response could have important implications for the treatment of human onchocerciasis and may go some way in explaining the long-term suppression of microfilariae observed in patients after treatment with ivermectin.

Human onchocerciasis is the result of chronic infection with the filarial nematode *Onchocerca volvulus* and affects around 18 million people, mainly in tropical Africa and Latin America (36). Infection is associated with a spectrum of dermal and ocular lesions resulting from the presence of first-stage larvae, or microfilariae, in the skin and eyes. The severity of the pathology has attracted a massive international effort to reduce the impact of onchocerciasis, initially through vector control and more recently by mass chemotherapy with the microfilaricidal drug ivermectin (22).

It is well established that the immune response to many parasitic helminths, such as *O. volvulus*, is directed by T lymphocytes, among which cells of the T helper (Th) 2 phenotype are usually predominant (27). Indeed, proliferative Th1 responses are generally down-regulated (10, 17, 35), and elevated serum immunoglobulin E (IgE) and eosinophilia are usually seen (19). However, changes in Th responses in patients with onchocerciasis following chemotherapy, as well as the development of a sustained Th1 parasite-specific response, have been demonstrated (29).

Investigations into functional immunity in onchocerciasis have been severely constrained by the host specificity of *O. volvulus* and the resultant dearth of appropriate animal models (4). In an attempt to circumvent this problem, we and others have devised mouse models based on specific phases of the life cycle in the definitive host that have been used to investigate immune mechanisms that are associated with the destruction of parasites in vivo (1, 6, 30, 32). Using a mouse model of host immunity to the skin-dwelling microfilariae in onchocerciasis, we have demonstrated that high levels of acquired resistance to *O. volvulus* or related *Onchocerca* spp. isolated from animals (e.g., *O. lienalis*) may be induced by prior sensitization with inoculations of living microfilariae (6, 32). In immunologically normal mice, the degree of inducible resistance is genetically determined (13) and is transferable with spleen cells, but not with serum, from immune donors to naive recipients (14). By in vivo depletion of selected cell populations, we have shown that clearance of the parasites is T-cell dependent and regulated by $CD4^+$ cells (14). Moreover, resistance to reinfection can be completely eliminated by neutralization of interleukin 5 (IL-5) (15).

Here we show that in the absence of cell reconstitution, severe combined immunodeficient (SCID) mice are unable to clear microfilariae from the skin. We confirm, by adoptive transfer of purified spleen cells into these animals, that clearance is dependent on $CD4^+$ T cells and is associated with high levels of circulating IL-5. Unexpectedly, unreconstituted SCID mice become highly resistant to reinfection following chemotherapy, manifested by reductions in parasite burdens equivalent to those observed in immunocompetent controls. This drug-induced resistance to microfilariae is clearly T cell independent and points to a hitherto unknown mechanism of host protection in mice. These findings are of interest because of the potential implications for our understanding of human onchocerciasis, and they may shed light on the long-term suppression of microfilariae observed in patients after treatment with ivermectin (16).

MATERIALS AND METHODS

Parasites. Microfilariae of *O. lienalis* were obtained from British cattle harboring natural infections. Parasites were recovered from the umbilical region of freshly slaughtered animals by the procedure of Bianco et al. (5) with minor modifications. Infected skin was surface sterilized with 70% ethanol, and superficial layers of the dermis were removed by scalpel. These were incubated overnight at room temperature in minimal essential medium (MEM; Gibco) supplemented with 10% newborn calf serum (Gibco) and antibiotics (200 U of penicillin per ml and 200 μg of streptomycin per ml; Gibco). Microfilariae released into the medium were concentrated by centrifugation (400 × *g* for 10 min) and passed through a filter of sponge to remove particulate host material.

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Parasites were resuspended in fresh supplemented MEM to the desired concentration.

Animals. BALB/c mice were obtained either from Olac (Bicester, United Kingdom) or from a breeding colony maintained at the Liverpool School of Tropical Medicine. C.B.17 *scid/scid* (SCID) mice (8) were obtained from either Bantam and Kingman (Hull, United Kingdom) or Liverpool School of Tropical Medicine stock (progeny of Bantam and Kingman breeding pairs). Males 8 to 10 weeks old were used throughout, unless otherwise stated. SCID mice with no detectable circulating IgG, determined by enzyme-linked immunosorbent assay (ELISA) (see below), were used in the experiments.

Infection of mice with microfilariae and recovery from the skin. Mice received a standard dose of 5,000 fresh (or 7,000 cryopreserved [18]) microfilariae in 0.2 ml of medium, administered subcutaneously at the nape of the neck. The recovery of live microfilariae from the ears was measured as an index of parasite establishment, as described by Townson and Bianco (31). Mice were killed by exsanguination under terminal anesthesia, and the ears were removed at the base. These were dispensed in pairs into sieves, finely minced in supplemented MEM, and incubated overnight at room temperature. Microfilariae were concentrated by centrifugation and counted in Sedgewick-Rafter chambers (Patterson, United Kingdom).

Cells. (i) Spleen cell preparation. Spleens were removed aseptically from naive BALB/c donors, and a single-cell suspension was prepared by passage through metal gauze into phosphate-buffered saline (PBS) supplemented with 2.5% fetal calf serum (FCS) and antibiotics (100 U of penicillin per ml and 100 μg of streptomycin per ml). Cells were washed (200 × *g* for 10 min at 4°C), and erythrocytes were lysed with Tris-buffered 0.83% ammonium chloride solution. Cells were washed again, and the pellets were resuspended into RPMI 1640 medium (Gibco) supplemented with 10% FCS and antibiotics. Viability was assessed by trypan blue dye exclusion, and cell concentration was determined with a hemocytometer.

(ii) Thymocyte preparation. Thymocytes were prepared from 6-week-old naive BALB/c donor mice. The thymus was removed aseptically from each animal, and a single-cell suspension was prepared by passage through metal gauze as described above. Cells were pooled and washed in supplemented RPMI 1640 medium as described above, and viability was assessed by trypan blue dye exclusion.

(iii) $CD4^+$-cell- and $CD8^+$-cell-depleted spleen cell populations. Prepacked glass bead columns (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) were set up according to the manufacturer's guidelines. Columns were washed with 15 ml of PBS containing 2% FCS, activated with a mixture of goat anti-mouse immunoglobulins (for surface immunoglobulin-positive [Ig^+]-cell depletion) and sheep anti-rat immunoglobulins (as a ligand for rat monoclonal antibodies, see below), and maintained for 2 h at room temperature. Spleen cells were incubated with either rat anti-mouse L3T4 (for $CD4^+$-cell depletion) or rat anti-mouse LYT2 (for $CD8^+$-cell depletion) for 30 min on ice, washed twice, and resuspended to 8×10^7 cells/ml in medium. Columns were washed with 20 ml of cell-free medium, and the column flow rate was adjusted to 6 drops per min. Cell suspension (1.5 ml) was added to each column, and the nonadherent cell population was collected in 15 ml of medium. Cells were washed and resuspended into supplemented RPMI 1640 medium, and their viability was assessed.

Immunofluorescence labelling. (i) Indirect method for $CD4^+$ and $CD8^+$ cells. Cells were washed in PBS containing 0.1% sodium azide (BDH, Poole, England). The supernatant medium was discarded, and cell pellets of 2×10^6 cells were resuspended in 15 μl of primary antibody diluted to 50 μg/ml. Rat monoclonal antibodies Y.T.S.191.2.1 (anti-CD4) and Y.T.S.169.4.2.1 (anti-CD8) were used (34), with normal rat IgG as a control. Cells were incubated for 1 h at 4°C, washed in PBS-azide, and resuspended in 15 μl of fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat immunoglobulins (Jackson Laboratories) for 30 min at 4°C. Cells were then washed in PBS-azide three times and resuspended in 0.4 ml of 1% paraformaldehyde (BDH). Fluorescence was assessed with a Becton-Dickinson FACScan.

(ii) Direct method for $Thy.1^+$ cells and B cells. FITC-conjugated anti-Thy.1 (Southern Biotechnology) and FITC-conjugated sheep anti-mouse immunoglobulins (courtesy of A. Lane, Regional Immunology, Southampton General Hospital) were used in direct staining of spleen cells. A total of 2×10^6 cells was incubated with antibody (15 μl at 50 μg/ml) at 4°C for 1 h, washed three times in PBS-azide, resuspended in 0.4 ml of 1% paraformaldehyde, and analyzed by FACScan.

ELISAs. (i) Detection of IgG. Total serum IgG was measured in all SCID mice before each experiment as a measure of "leakiness." Only IgG-negative SCID mice were used in experiments. At the end of each experiment, serum IgG was measured again. ELISA plates (Nunc Maxisorp) were coated with 100 μl of goat anti-mouse IgG (Z420; Dako) diluted 1:1,000 in 0.05 M carbonate buffer, pH 9.6, overnight at room temperature. Plates were washed three times for 10 min each time in PBS containing 0.1% Tween 20 (Sigma) and blocked with 120 μl of PBS containing 2% bovine serum albumin (BDH) for 1 h at room temperature. After a second round of washing, 100-μl serum samples, at 1/100 and 1/400 dilutions in wash buffer, were added and the plates were incubated for 1 h at 37°C. Pooled normal mouse serum, similarly diluted, was used as a positive control. Plates were washed, and 100 μl of peroxidase-conjugated rabbit anti-mouse IgG (P260; Dako), at a 1/1,000 dilution, was added for 3 h at room temperature. After wells were washed, 50 μl of orthophenylene diamide substrate solution was added to each well, and the reaction was stopped after 10 min with 50 μl of 3 M sulfuric acid. Plates were read at 492 nm on a Dynatech plate reader, and optical densities of >0.1 were considered positive.

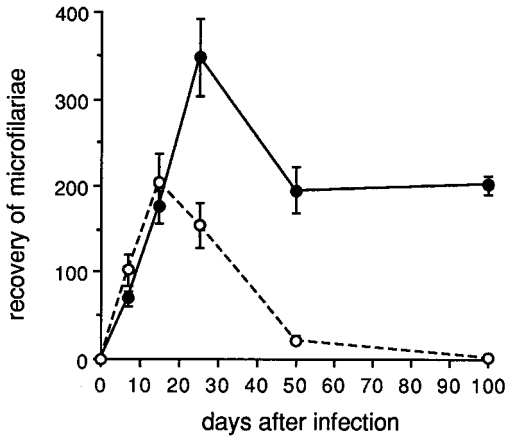

FIG. 1. Mean (± standard error) parasite recoveries from SCID (●) and BALB/c (○) mice at various times following infection with *O. lienalis* microfilariae ($P < 0.01$, day 25; $P < 0.001$, day 50; $P < 0.001$, day 100).

(ii) Detection of IL-5 and IFN-γ. IL-5 and gamma interferon (IFN-γ) were detected by sandwich ELISA as previously described by Else and Grencis (11). Monoclonal antibodies 5P8.23 and TRFK-4 (DNAX, Inc., Palo Alto, Calif.) were used for IL-5 detection, and R4-6A2 (E. Havell) and XMG1.2 (DNAX) were used for IFN-γ detection. ELISA plates were coated overnight at 4°C with 50 μl of capture antibody diluted in PBS. One hundred fifty microliters of blocking solution (10% FCS in PBS) was then added to each well, and plates were incubated for 30 min at 37°C. Plates were washed briefly three times, followed by two 3-min washes in PBS containing 0.1% Tween 20. Reference standards for each of the cytokines were prepared in RPMI 1640 supplemented with 10% FCS. Fifty microliters of each standard or sample was transferred to the test plates and incubated for 2 h at room temperature. Plates were washed, and 50 μl of biotinylated second antibody diluted in PBS-Tween containing 0.1% bovine serum albumin was added for 1 h at room temperature. Plates were washed, and 75 μl of streptavidin-peroxidase (Boehringer-Mannheim) diluted 1/1,000 was added for 1 h at room temperature. Plates were washed, and 100 μl of substrate solution [2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS) citrate buffer with hydrogen peroxide; Sigma] was added. Plates were read at 405 nm on a Dynatech ELISA plate reader.

Statistics. Parasite recoveries from groups of infected mice were analyzed by Student's unpaired *t* test, and *P* values of <0.05 were considered statistically significant.

RESULTS

SCID mice cannot clear primary-infection microfilariae. Thirty SCID and 30 BALB/c control mice were inoculated with 5,000 *O. lienalis* microfilariae. Six mice of each strain were sacrificed on days 7, 15, 25, 50, and 100 postinfection (Fig. 1). The survival of microfilariae in SCID mice was significantly extended compared with that in BALB/c controls (day 25, $P < 0.01$; day 50, $P < 0.001$; day 100, $P < 0.001$); the parasite persisted for at least 100 days, at which point the experiment was terminated. By this time, BALB/c mice had virtually cleared their microfilariae, as predicted from the results of previous experiments (14, 15).

Reconstitution of SCID mice with spleen cells aids parasite clearance. Eighteen SCID mice and 12 BALB/c controls were randomly divided into groups of 6 mice. One group of SCID mice received 4×10^7 naive BALB/c whole spleen cells per recipient, while another group received 4×10^7 naive BALB/c thymocytes per recipient, by intravenous injections. Cell dosage was based upon the proven effectiveness of adoptive transfers in excess of 3×10^6 cells per recipient in previous experiments (9). All groups of mice were challenged with 5,000 *O. lienalis* microfilariae approximately 24 h later. Six BALB/c con-

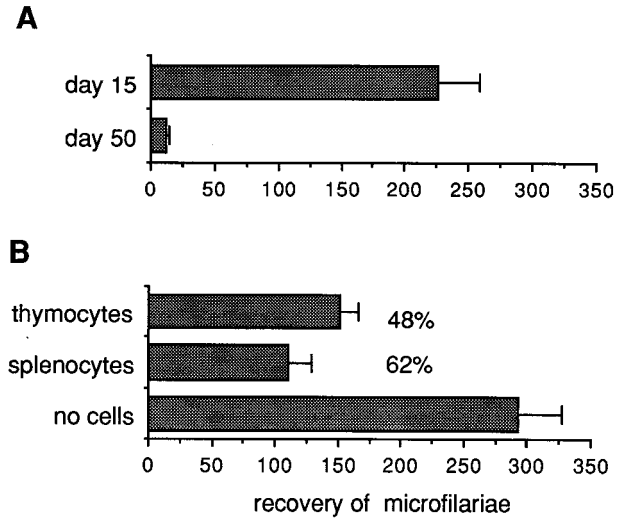

FIG. 2. Mean (± standard error) recoveries of *O. lienalis* microfilariae from SCID mice, 50 days postinfection, following reconstitution with thymocytes or splenocytes, compared with recovery in unreconstituted animals (B). Parasite recoveries from immunocompetent BALB/c control mice sacrificed 15 or 50 days postinfection are shown for comparison (A). Adoptive transfers with either cell population conferred protection against microfilariae as shown by comparison with unreconstituted SCID mice. Percent protection (reduction in the numbers of microfilariae recovered compared with numbers in the unreconstituted control group) is indicated ($P < 0.01$ in each case).

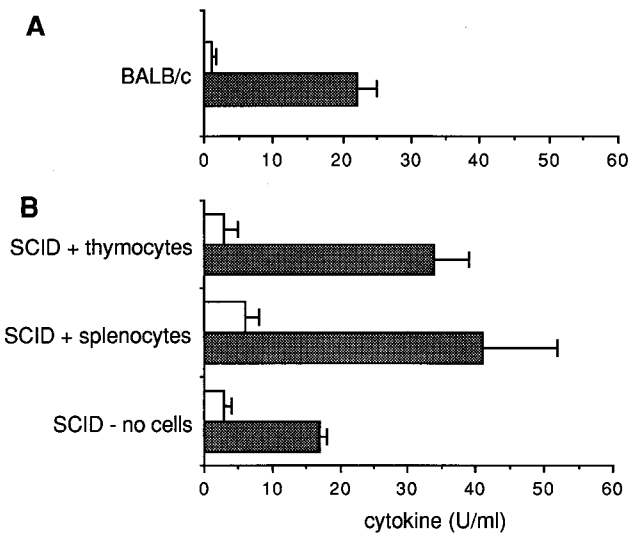

FIG. 3. Mean (± standard error) levels of IL-5 (shaded bars) and IFN-γ (white bars) in the sera of SCID mice following reconstitution with either thymocytes or splenocytes and infection with *O. lienalis* microfilariae (B). Cytokine levels in the sera of immunocompetent BALB/c control mice are shown for comparison (A). Sera were collected 50 days postinfection.

trol mice were sacrificed 15 days postchallenge in order to monitor parasite establishment. The remaining mice were sacrificed on day 50 to discriminate between immune and susceptible animals on the basis of parasite recoveries, as illustrated in the previous experiment. Protection conferred by transferred cells was calculated by the comparison of parasite recoveries from reconstituted versus unreconstituted SCID mice (Fig. 2).

At 50 days postchallenge, unreconstituted SCID mice showed no protection against microfilariae and retained high parasite numbers (Fig. 2B) compared with those in BALB/c controls (Fig. 2A). Reconstituted SCID mice, however, did show significant levels of protection, the extent of which was dependent on the donor cell population. BALB/c spleen cells and thymocytes resulted in reductions in parasite recoveries (relative to unreconstituted controls) of 62% ($P < 0.01$) and 48% ($P < 0.01$), respectively (Fig. 2B). The composition of donor cell populations was assessed by fluorescence staining for surface immunoglobulin (B cells) and Thy.1 (T cells). The proportions of B and T cells in normal BALB/c spleen cells were 47 and 37%, respectively. The proportion of BALB/c donor thymocytes was 85% Thy.1$^+$, with 2% contaminating Ig$^+$ cells.

Levels of IL-5 and IFN-γ were measured in the sera of reconstituted and unreconstituted SCID mice on day 50 postchallenge (Fig. 3). Reconstitution of SCID mice by either whole spleen cell or thymocyte transfer boosted the level of IL-5 ($P = 0.05$ in each case) but did not cause an increase in the levels of IFN-γ.

Requirement for CD4$^+$ cells in the expression of protection against microfilariae in reconstituted SCID mice. Twenty-four SCID and 12 BALB/c mice were randomly divided into groups of 6. Three SCID groups received by intravenous injection either (i) 4×10^7 whole spleen cells, (ii) 1×10^7 CD8$^+$-cell-depleted spleen cells, or (iii) 0.6×10^7 CD4$^+$-cell-depleted spleen cells from naive BALB/c donors. The numbers of cells used in each category reflected their abundance in the spleens of donor mice. All animals were challenged 24 h later with 7,000 cryopreserved microfilariae. Six BALB/c control mice were sacrificed 15 days postchallenge to monitor parasite establishment. The remaining mice were sacrificed 50 days postchallenge, and parasite recoveries were measured.

Reconstitution of SCID mice with either whole spleen cells or CD8$^+$-cell-depleted spleen cells allowed the development of protection against microfilariae (Fig. 4B). Reductions in parasite recoveries after adoptive transfer measured at 50 days postchallenge were 45% ($P < 0.01$) and 35% ($P < 0.05$) for whole spleen cells and CD8$^+$-cell-depleted spleen cells, respectively (Fig. 4B), compared with unreconstituted SCID mouse controls. CD4$^+$-cell-depleted spleen cells did not significantly affect the survival of microfilariae, although the possibility that engraftment of the residual CD8$^+$ cells was too transient to affect the recoveries of microfilariae on day 50 cannot be ruled out.

The composition of donor cell populations was assessed by fluorescence-activated cell sorter (FACS) analysis. Whole spleen cells were 46% Ig$^+$, 44% CD4$^+$, and 22% CD8$^+$; the CD8$^+$-cell-depleted population contained 64% CD4$^+$ cells, with 2% contaminating CD8$^+$ cells; and the CD4$^+$-cell-depleted population comprised 45% CD8$^+$ cells, with 2% CD4$^+$ contaminating cells. In the last two preparations, Ig$^+$ cells were reduced to 1 and 3%, respectively. We conclude that the depletions of CD4$^+$ and CD8$^+$ cells in the donor splenocyte population were sufficient to establish the relative importance of each cell type in the expression of host protection in reconstituted SCID mice.

Exposure of SCID mice to a drug-abbreviated primary infection induces resistance to reinfection. Twelve SCID and 12 BALB/c mice were randomly divided into groups of 6. One group of each strain received a primary inoculation of 5,000 microfilariae and the mice were rested for 100 days. Because SCID mice are unable to clear their parasites (Fig. 1), the microfilaricidal drug ivermectin was administered on day 100 postinfection. To control for drug exposure, all mice received ivermectin at the same time, at a dosage (0.2 mg/kg) previously

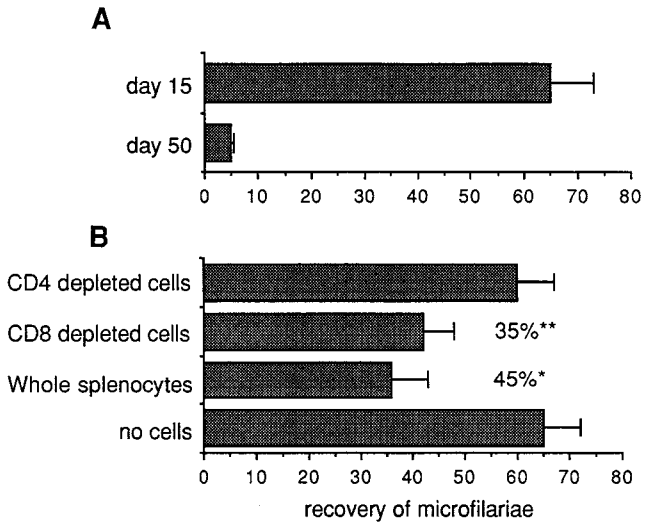

FIG. 4. Mean (± standard error) recoveries of *O. lienalis* microfilariae from SCID mice, 50 days postinfection, following reconstitution with $CD4^+$-cell-depleted, $CD8^+$-cell-depleted, or whole unfractionated spleen cells (B). Included in panel B are results from a control group of unreconstituted SCID mice. Parasite recoveries from BALB/c control mice sacrificed 15 or 50 days postinfection are shown for comparison (A). The adoptive transfer of whole spleen cells or $CD8^+$-cell-depleted splenocytes (but not $CD4^+$-cell-depleted splenocytes) provided significant protection against infection. Percent protection (reduction in the numbers of microfilariae recovered compared with numbers in the unreconstituted control group) is indicated (**, $P < 0.05$; *, $P < 0.01$).

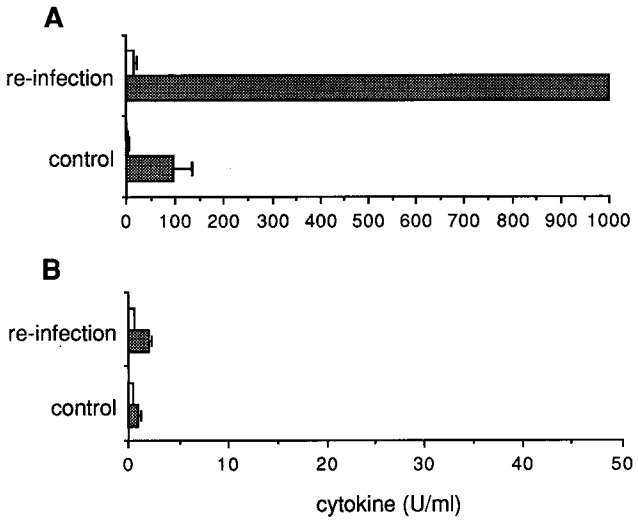

FIG. 6. Mean (± standard error) levels of IL-5 (A) and IFN-γ (B) in the sera of SCID (white bars) and BALB/c (shaded bars) mice, 15 days after secondary (reinfection) or primary (challenge control) infections with *O. lienalis* microfilariae.

shown to remove microfilariae from immunocompetent animals within 24 h (7). This dose was known to exert no residual microfilaricidal activity 30 days after treatment (7). Accordingly, mice were challenged 30 days later with 5,000 microfilariae, and parasite recoveries were measured 15 days after challenge.

Resistance to challenge with microfilariae occurred in both SCID and BALB/c mice primed by drug-abbreviated infections (Fig. 5). Equivalent reductions in recoveries of microfilariae were observed in the two groups (70% reduction in recoveries compared with challenge control groups, $P < 0.05$ and $P < 0.01$ for SCID and BALB/c mice, respectively) (Fig. 5A). This experiment was repeated with similar results (63 and 82% reductions, $P < 0.01$ and $P < 0.001$ for SCID and BALB/c mice, respectively; data not shown).

The cytokines IL-5 and IFN-γ were measured in the sera of experimental mice (Fig. 6). While BALB/c mice clearly showed a preferential increase in IL-5 (Fig. 6A) over IFN-γ (Fig. 6B), the levels of both cytokines in the SCID mouse sera remained low. Measurement of serum IgG in individual animals postmortem showed that no appreciable antibody leakiness had occurred in the SCID mice during the course of the experiment (data not shown).

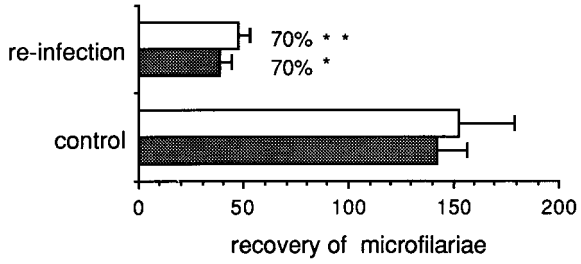

FIG. 5. Mean (± standard error) parasite recoveries from BALB/c (shaded bars) and SCID (white bars) mice following an ivermectin-abbreviated primary infection and reinfection with *O. lienalis* microfilariae. Parasite recoveries were measured 15 days after challenge. Mice of all groups received ivermectin at the same time and dosage to control for drug exposure and clearance. Percent protection (reduction in the numbers of microfilariae recovered from reinfected mice compared with numbers in their respective challenge control group) is indicated (**, $P < 0.05$; *, $P < 0.01$).

DISCUSSION

SCID mice are unable to clear *O. lienalis* microfilariae following a primary infection and instead harbor large parasite numbers over a prolonged period of time. Reconstitution of SCID mice with T cells and in particular $CD4^+$ cells from naive BALB/c donors restored their ability to clear microfilariae, as observed in immunocompetent BALB/c controls. Measurement of Th1 and Th2 representative cytokines in the sera of reconstituted animals demonstrated relatively high levels of IL-5 and low levels of IFN-γ, supporting the case that a Th2 response is involved in parasite killing. These results are in accordance with data obtained with immunocompetent mice, which show that $CD4^+$ Th2 cells and IL-5 are key elements in the destruction of *O. lienalis* microfilariae in mice (14, 15).

Resistance to reinfection in SCID mice following a drug-abbreviated primary infection appears to represent a mechanism of host protection distinct from that seen in immunocompetent mice. This resistance occurs in the absence of T cells and is associated with relatively low levels of both IL-5 and IFN-γ. How treatment with ivermectin results in activation of a protective T-cell-independent response is not clear, but it may involve events triggered by the sudden release of parasite products from dying microfilariae. Importantly, this state of activation is maintained for at least 30 days after the parasites have been destroyed, indicating that it goes beyond a transient elevation in phagocytic cell functions.

Ivermectin has been shown to kill *Onchocerca* microfilariae in vitro (33), most probably by interference with nerve signal transmission (2). In trials of the drug in humans, it has been reported to have sustained microfilaricidal effects that are not easily explained, because ivermectin is very rapidly eliminated

from the tissues. It has been proposed that ivermectin may achieve this sustained activity through an effect on adult female worm fecundity (26). Elevated levels of plasma elastase in onchocerciasis patients treated with ivermectin have also been demonstrated, suggesting an alternative or supplementary mechanism of antiparasitic activity that may involve neutrophil activation (24). It has also been observed that there is an increase in the acute-phase reactants C-reactive protein and interleukin 6, which may underlie adverse responses to treatment (23).

Soboslay and colleagues have shown that treatment of onchocerciasis patients with ivermectin restores the cellular immune response to parasite antigens, activating a parasite-specific Th1-type response (28, 29). This may mirror the cellular immune response seen in putatively immune individuals from areas in which onchocerciasis is endemic (12). Such changes have also been described following chemotherapy of human lymphatic filariasis, with improved cellular proliferative responses to parasite antigens coupled with production of IFN-γ (25). The common denominator of these reports is that chemotherapy leads to the emergence of Th1 responses that appear to be suppressed during patent infections.

The results from SCID mice presented here suggest an additional effect of ivermectin treatment on host defenses, which thus far has not been considered in these infections. Interestingly, other reports of parasitic infections in SCID mice have described both innate and specific immune protection. McDonald and Bancroft (20) have shown that SCID mice reconstituted with lymphocytes exhibit a $CD4^+$ Th1 protective response against the protozoan parasite *Cryptosporidium parvum* similar to that seen in immunocompetent control animals. However, in the absence of reconstitution, SCID mice mount a transient protective response that is associated with IFN-γ production by natural killer (NK) cells (3, 20, 21). The relatively low serum IFN-γ levels in unreconstituted SCID mice expressing resistance to *Onchocerca* microfilariae might argue against the involvement of NK cells in the system presented here. However, cellular activity in the draining lymph nodes of these animals was not investigated, so the possibility of NK cell involvement cannot be excluded. The exact mechanism of drug-assisted resistance to microfilariae in the SCID mouse therefore remains to be determined.

In summary, this report illustrates the importance and complementary roles of T-cell-dependent and T-cell-independent responses in the regulation of a tissue helminth by the immune system of the host. While the former is defined as a classical Th2 response involving $CD4^+$ cells and IL-5, the latter is induced by chemotherapy and functions in the total absence of T cells. The underlying mechanisms of resistance induced by drug-abbreviated infection need to be clarified so that the predictive value of these observations for human onchocerciasis can be explored. This information may be of value in the design of sustained ivermectin treatment campaigns for the control of onchocerciasis, which the World Health Organization and others are currently promoting throughout the foci of endemicity in Africa and Latin America (22).

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