Characterization of Rat T Helper Cell Clones Specific for 
Bacteroides gingivalis Antigen

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During the past several years, much interest has been directed towards delineating and characterizing different subsets of T helper (Th) cells in order to understand their roles in immune processes. In this study, we report the generation of antigen-specific rat Th cell clones and their characterization in terms of phenotype, function, and lymphokine production. The clones were derived by culturing purified splenic T cells from rats immunized with the pathogen Bacteroides gingivalis with equivalent numbers of irradiated spleen cells from nonimmune rats and B. gingivalis whole-cell antigen. The clones required antigen stimulation but not exogenously added interleukin-2 for growth and were maintained in culture for approximately 6 months. The cloned T cells proliferated in response to the mitogen concanavalin A and to B. gingivalis whole-cell antigen but not to other microbial antigens. Phenotypic characterization of the cloned T cells for cell surface markers demonstrated that these cells were OX19+ W3/25+ OX8− OX22− and therefore probably represented a mature subpopulation of CD4+ Th cells. These cloned T cells were positive for interleukin-2 receptor expression. Culture supernatants from the Th cell clones which were collected at various times after antigen stimulation exhibited low interleukin-2 activity and high gamma interferon activity. This in vitro study provides evidence of a rat Th cell subset that could represent an important population in regulating immune responses to microbial antigens.

Periodontitis is an inflammatory disease that involves the destruction of oral tissues surrounding the teeth, including attachment fibers and the alveolar bone (14). The etiology of this disease is attributed to the presence of certain species of bacteria, among which the black-pigmented Bacteroides gingivalis has been most strongly implicated (12, 14). The immunopathologic mechanisms involved in this disease are not totally understood, although the interaction between bacterial antigens and the T lymphocytes in the inflamed oral lesion has been suggested to be of importance in the pathogenesis of the disease in humans (11, 27) and in the experimental rat model (32). Therefore, to help delineate the T-cell events involved in the regulation of this disease, it is important to determine the functional differences and modes of activation of T-cell subsets which may be involved in mediating the disease.

With the discovery by Mosmann and co-workers (4, 21, 22) of two different types of cloned murine T helper (Th) cells (Th1 and Th2), a foundation was set to help determine the contribution of these and potentially other types of Th cell subsets in actual in vivo immune responses. The two subpopulations of murine Th cell clones are primarily divided by the type of lymphokines they produce. Th1 cells are characterized by the secretion of interleukin-2 (IL-2), gamma interferon (IFN-γ), and lymphotoxin, whereas Th2 cells secrete interleukin-4 (IL-4) (but not IL-2) and interleukin-5 (IL-5). The function of these two subsets relates to the lymphokines they produce. Th2 cells produce the major helper factors for B-cell responses, while factors produced by Th1 cells are mediators of delayed-type hypersensitivity reactions (4, 21, 22).

Despite the extensive work in the murine system, studies in other species, such as human and rat, have been limited due to difficulty in generating T-cell clones. Therefore, other investigations have placed emphasis on determining whether antibodies to the leukocyte-common antigen (L-CA), a surface antigen expressed on most hematopoietic cells, would be useful in differentiating Th cell subsets. Antibodies to the L-CA have been shown to divide Th cells into subsets in the human (17, 18), mouse (3), and rat (1) systems; however, it is unclear whether this division identifies separate lineages, activation states, or functional potentials.

Studies by Mason and colleagues (1, 23, 24) have shown that the monoclonal antibody (Mab) MRC OX22 binds to the high-molecular-weight form of the CD45 antigen and divides the rat Th cell population into CD4+ OX22+ cells and CD4+ OX22− cells. Although a correlation between the expression of the OX22 marker and the synthesis of lymphokines has not been defined, it has been reported that CD4+ cells produce IL-2, with the CD4+ OX22+ cells producing higher amounts than the CD4+ OX22− cells (1). It also has been shown that CD4+ OX22+ cells proliferate in response to allogeneic cells and to mitogens (1), whereas CD4+ OX22− T cells provide B-cell help for secondary immune responses (1, 23, 29).

In this article, we report the generation of rat Th cell clones specific for B. gingivalis and provide information on their phenotype and the lymphokines they secrete.

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MATERIALS AND METHODS

Rats. Inbred Fischer female rats [CDF (F-344)CrlBR; originally obtained from Charles River Breeding Laboratories, Wilmington, Mass.] used in these studies were derived from a breeding colony maintained in the University of Alabama at Birmingham Gnotobiotic Rat Facility. All rats were maintained in horizontal laminar flow hoods and provided food and water ad libitum.

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Antigen preparation. *B. gingivalis* ATCC 33277 was grown at 37°C under anaerobic conditions as described before (30, 31). The organisms were harvested by centrifugation (10,000 × g), washed in sterile phosphate-buffered saline (PBS; 0.01 M, pH 7.4) and suspended in sterile PBS containing 0.02% sodium azide. The *B. gingivalis* whole cells (WC) were maintained at 4°C until used to immunize animals or for use in vitro cultures. Other antigens used were WC of *Streptococcus mutans* and *Salmonella typhimurium* prepared as described previously (5, 16).

Immunization. Female rats (8 to 12 weeks of age) were given a single intraperitoneal injection (0.5 ml) of *B. gingivalis* ATCC 33277 WC (3 × 10⁸ cells per ml) in complete Freund adjuvant. Six to 8 weeks later, rats were given an intravenous injection (0.25 ml) of *B. gingivalis* WC in pyrogen-free saline (10⁶ cells per ml), and then 3 days later, rats were killed for the isolation and purification of splenic T lymphocytes.

Reagents. Mouse MAbs used in this study were OX19, specific for rat T lymphocytes (CD5); W3/25, specific for the rat T helper/inducer subpopulation (CD4); OX8, specific for the cytotoxic and/or suppressor populations (CD8); OX39, specific for the rat IL-2 receptor; and OX22, which recognizes the high-molecular-weight form of the L-CA (CD45) (kindly provided by Allan F. Williams, Medical Research Council Cellular Immunology Unit, Oxford University, Oxford, England). A MAb (MAR 18.5) specific for rat kappa chain (15) was used as our B-cell-specific reagent. Each of these reagents was available as either fluorescein isothiocyanate or biotin conjugates. Avidin-phycocerythrin was purchased from Southern Biotechnology Associates, Inc., Birmingham, Ala. Cell populations were phenotypically characterized following incubation of cell samples with various combinations of the MAbs and analysis with a FACStar fluorescence-activated cell sorter (FACS; Becton Dickinson, Mountain View, Calif.).

Concanavalin A (Con A)-stimulated rat spleen cell culture supernatant (Con A-SN) was prepared as described previously (7, 19), filter sterilized, and stored at −70°C until used.

Purification of splenic T-lymphocyte populations. The procedures used for the purification of splenic lymphoid cells were similar to those reported previously (7). Rats were killed by carbon dioxide asphyxiation, their spleens were aseptically removed, and single-cell suspensions were prepared by mechanically dispersing the tissue through sterile wire mesh into RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.). Erythrocytes were lysed with ammonium chloride (ACK) buffer. The cells were washed, suspended in warm (37°C) RPMI 1640 supplemented with 5% fetal calf serum (FCS), and passed through a Sephadex G-10 (Pharmacia, Piscataway, N.J.) column equilibrated with the same warm medium. The eluted cell population was depleted of B cells by panning (39) on plastic petri plates (15 by 100 mm; Falcon Labware, Oxnard, Calif.) coated with immunoglobulin G rabbit anti-rat F(ab′)₂ (7, 19). After incubation for 90 min at 4°C, the nonadherent cell population was collected, washed twice with RPMI 1640, and suspended in RPMI 1640 supplemented with 2 mM L-glutamine, 10 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), 100 U of penicillin per ml, 10 µg of streptomycin per ml, 20 µg of gentamicin per ml (incomplete medium), 5 × 10⁻⁵ M 2-mercaptoethanol and 10% FCS (complete medium) for in vitro culture. The resulting cell population was >97% T cells (OX19⁺) and >98% viable as determined by trypsin blue exclusion.

Generation of T-cell clones. Purified splenic T cells (5 × 10⁵ per well) from rats immunized with *B. gingivalis* antigen were cultured in complete medium in 24-well tissue culture plates together with *B. gingivalis* WC (5 × 10⁸ per well) and irradiated (1,500 rads with a 3²⁵Cs source; Gamma Cell 40, Atomic Energy of Canada Ltd., Ontario, Canada) nonimmune spleen cells (5 × 10⁵ per well) which had been treated with ACK buffer and passed over a Sephadex G-10 column (designated irradiated spleen cells). Cultures were incubated at 37°C in a humidified atmosphere of 10% CO₂ in air (standard incubation conditions unless otherwise stated). Antigen and irradiated spleen cells were added to cultures every 4 to 7 days. After 4 to 6 weeks, T cells were separated on a Ficol-Hypaque density gradient and cultured in complete medium supplemented with Con A-SN at 0.3 or 0.1 cells per well with irradiated spleen cells and antigen (each at 5 × 10⁶ per well) in 96-well round-bottomed plates (Corning 25850; Corning Glass Works, Corning, N.Y.). After 7 to 14 days, wells exhibiting clonal growth were expanded into 24-well plates. The T-cell clones were maintained by stimulation with antigen in the presence of irradiated spleen cells. This procedure has been used to generate antigen-specific rat T-cell clones to different microbial WC (unpublished data). In the present study, 15 clones with antigen specificity to *B. gingivalis* WC were generated, of which T cells from 5 clones were extensively characterized. The characteristics of these cloned T cells were similar, and therefore the results presented are from clone 2 and are representative of all clones analyzed.

Proliferative assay. Cloned T cells were cultured in 96-well flat-bottomed plates (Falcon) in quadruplicate at 4 × 10⁵ cells per well in incomplete medium supplemented with 1% FCS. Cultures were incubated at 37°C, 5% CO₂ with irradiated spleen cells (2 × 10⁵ per well) alone or with various concentrations of Con A (1.0 or 0.1 µg/ml of culture), *B. gingivalis*, *S. typhimurium*, or *S. mutans* WC (10⁵ to 1 WC/ml of culture) for 72 h. Cultures were pulsed with [³H]thymidine (Amersham Corp., Arlington Heights, Ill.) (0.5 µCi per well) during the last 18 h of incubation. Cells were harvested onto a glass fiber filter with a MASH 1 T-cell harvester (Microbiological Associates, Walkersville, Md.), and the amount of [³H]thymidine incorporation was measured in a liquid scintillation counter.

IL-2 assay. Culture supernatants (SN) from the T-cell clones were assayed for IL-2 activity on the HT-2AB cell line (kindly provided by H. G. A. Bouwer, Providence Medical Center, Portland, Oreg.) as previously described (2, 37). The HT-2AB cells proliferate in the presence of IL-2 but do not respond to IL-4 (H. G. A. Bouwer, personal communication). Twofold serial dilutions of SN were added (50 µl per well) to quadruplicate wells of 96-well flat-bottomed plates (Falcon) containing 5 × 10⁴ HT-2AB cells per well in 100 µl of complete medium supplemented with 1 mM sodium pyruvate. Plates were incubated at 37°C in an atmosphere of 10% CO₂ in air for 24 h, and the cultures were assessed for proliferation by the MTT colorimetric assay (see below). Dilutions of Con A-SN and of recombinant IL-2 (13) were included in all assays as positive controls. Cultures consisting of HT-2AB cells only served as the negative (baseline) control.

IFN-γ assay. Production of IFN-γ by the cloned T cells was assessed by inhibition of growth of the IFN-γ-sensitive WEHI-279 cell line (2, 25). WEHI-279 cells were cultured in 96-well flat-bottomed plates at a concentration of 5 × 10⁵ cells per well (50 µl) in incomplete medium supplemented with 5 × 10⁻⁵ M 2-mercaptoethanol, 1 mM sodium pyruvate, and 5% FCS. Twofold serial dilutions of SN from T-cell
clones were added (50 μl) to quadruplicate wells. Cultures were incubated for 72 h and then assessed for proliferative activity by the MTT colorimetric assay (see below). The monoclonal anti-rat IFN-γ antibody DB1 (35) used to confirm the specificity of this assay was kindly provided by Roland Jonsson (University of Goteborg, Goteborg, Sweden). Dilutions of Con A-SN and of recombinant IFN-γ (Genzyme Corp., Boston, Mass.) were included in all assays as controls. Cultures consisting of WEHI-279 cells only served as the positive control. Wells with medium only served as the negative control.

MTT assay. The MTT assay was performed by a modification of the method described by Mosmann (20). Following incubation of cultures, stock MTT [3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide; Sigma Chemical Co., St. Louis, Mo.] solution (5 mg/ml of PBS) was added to cultures (10 μl per well). Plates were incubated for an additional 4 h at 37°C, and then acid-isopropanol (0.04 N HCl in isopropanol) was added to wells (150 μl per well). The content of the wells was mixed thoroughly to ensure that all crystals were dissolved, and the color change (representing cell proliferation) was read within 1 h at 590 and 650 nm with a Vmax microplate reader (Molecular Devices Corporation, Palo Alto, Calif.).

RESULTS

Phenotypic characterization of rat splenic lymphoid cell populations. The surface markers expressed by the unfractionated rat spleen cell population and the enriched subpopulations employed in this study were determined by flow cytometry. After passage of spleen cells through a Sephadex G-10 column, approximately equal proportions of the cells (~45%) stained with the MAR 18.5 (Slg+) or with OX19 (CD5) MAb (Fig. 1). Further analysis showed that nearly half of the CD5+ T cells were CD4+ Th cells as indicated by staining with the MAb W3/25. The CD4+ population could be divided into two subsets with the MAb OX22, as has been reported previously (1, 23, 24), with approximately half of the CD4+ spleen cell population being CD4+ OX22- (54%) and the other half being CD4+ OX22+ (46%).

After panning of the cells on anti-rat F(ab')2-coated plates (see Materials and Methods), the resulting nonadherent cell population was again analyzed by FACS (Fig. 1). This enriched subpopulation consisted of CD5+ T cells, and contained no Slg+ cells, as established with the MAR 18.5 MAb. By comparing the contours obtained with the purified T cells with those obtained with the G-10-passed cell preparation, the proportions of CD4+ T cells and of CD4+ OX22- and CD4+ OX22+ cells were found to be similar. The small population of OX19+ cells (~10%) in the anti-F(ab')2 nonadherent fraction stained with the MAb OX8 (data not shown).

Antigen specificity of T-cell clones. G-10-passed and anti-F(ab')2-nonadherent T cells from B. gingivalis-immunized rats were used to establish antigen-specific T-cell clones. Growth of the T-cell clones was dependent upon the presence of both irradiated spleen cells and B. gingivalis WC, but not exogenously added IL-2. The clones were maintained in culture for a period of approximately 6 months. Five T-cell clones were extensively characterized in this study. Since the characteristics of these cloned T cells were similar, only the results from clone 2 are presented and are representative of the other clones analyzed.

When the cloned T cells were incubated without irradiated spleen cells or without B. gingivalis WC or with other

FIG. 1. Analysis of cell surface markers on rat splenic lymphoid cell populations by flow cytometry. Rat spleen cells were initially passed through a Sephadex G-10 column (left panels) and then, following panning on anti-rat F(ab')2-coated plates, the nonadherent cell population was collected (right panels). Samples (2 × 10^5 cells) of the G-10-passed and of the nonadherent cell preparations were stained with fluorescein isothiocyanate-labeled anti-rat F(ab')2, reagent and/or biotinylated reagent and avidin-phycocerythrin. Profiles are representative of four different analyses of similarly prepared cell populations. The top two sets of panels are profiles of cells stained with a single reagent (indicated on the abscissa). The bottom two sets of panels are profiles of cells stained with two different reagents (indicated on the abscissa and ordinate).
TABLE 1. Proliferative responses of cloned T cells to B. gingivalis WC antigen

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Conc (per ml of culture)</th>
<th>[3H]thymidine uptake&lt;sup&gt;b&lt;/sup&gt; (cpm/culture)</th>
</tr>
</thead>
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<tr>
<td>B. gingivalis WC</td>
<td>10⁷</td>
<td>5,420</td>
</tr>
<tr>
<td></td>
<td>10⁶</td>
<td>8,835</td>
</tr>
<tr>
<td></td>
<td>10⁵</td>
<td>3,241</td>
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<td>10⁴</td>
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<td></td>
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<td>2,095</td>
</tr>
<tr>
<td></td>
<td>10¹</td>
<td>4,398</td>
</tr>
<tr>
<td></td>
<td>10⁰</td>
<td>1,256</td>
</tr>
<tr>
<td>Con A</td>
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<tr>
<td></td>
<td>0.1 µg</td>
<td>4,326</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>520</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cloned T cells (4 × 10⁵ cells per well) were cultured with or without stimulant for 72 h and pulsed with [3H]thymidine during the last 18 h of incubation (see Materials and Methods). The amount of [3H]thymidine uptake in cultures incubated with various concentrations of S. mutans or S. typhimurium WC (10⁶ to 1/ml of culture) was less than 1,000 cpm per culture.

<sup>b</sup> Values are the mean of quadruplicate cultures per experiment with an SEM of less than 15% and are representative of three separate experiments.

Microbial (S. mutans or S. typhimurium) WC, essentially no growth occurred. The cloned T cells proliferated in response to the mitogen Con A and to B. gingivalis WC (Table 1). A concentration of 10⁶ B. gingivalis WC per ml induced a peak proliferative response. The response observed in cultures containing an estimated 10 B. gingivalis WC per ml may be to a soluble cell wall component present in the solution; however, further studies are required to clarify this observation. No thymidine uptake was seen in cultures of cloned T cells incubated with S. mutans or S. typhimurium WC over a wide range of doses (10⁴ to 1 bacterial WC per ml of culture; data not shown).

Phenotypic analysis of rat T-cell clones. Analysis of the T-cell clones for surface markers by flow cytometry revealed that they were OX19<sup>+</sup> W3/25<sup>+</sup> (CD5<sup>+</sup> CD4<sup>+</sup>) and thus of the Th cell phenotype (Fig. 2). These cells did not stain with the MAB MAR 18.5 (Slg<sup>+</sup>) or OX8 (CD8<sup>+</sup>). Furthermore, these cells did not express the high-molecular-weight form of the L-CA recognized by the MAb OX22, but did express IL-2 receptors as determined by using the MAb OX39.

Lymphokine production by T-cell clones. The HT-2AB cell line, which is dependent on the presence of IL-2 for growth but does not proliferate in the presence of IL-4 (data not shown), was used to assay SN from the T-cell clones for IL-2 activity (Fig. 3). A low but detectable level of IL-2 activity (<1 U/ml) was reproducibly found in the SN from the T-cell clones (Fig. 3). A similar low level of activity was detected in SN from cloned T cells at various times following antigen stimulation (data not shown). These cells were, however, expressing IL-2 receptors, as shown by FACS analysis with the OX39 MAb (Fig. 2). The Con A-SN used as a positive control exhibited good IL-2 activity in this assay (Fig. 3). SN from cultures consisting of irradiated spleen cells with or without antigen did not exhibit IL-2 activity (data not shown).

Although the SN from the T-cell clones contained relatively low levels of detectable IL-2 activity, they did contain high levels of IFN-γ activity (~500 U/ml) as determined by using the IFN-γ-sensitive WEHI-279 cell line (Fig. 4). Ninety-eight percent of this activity was abrogated by the addition of the anti-rat IFN-γ MAb DB1 (2.5 µg per well) to the cultures. SN from cultures consisting of irradiated spleen cells with or without antigen did not exhibit IFN-γ activity (data not shown).

**DISCUSSION**

An objective of the experiments described in this report was to generate and characterize cloned rat T cells with antigen specificity to the oral pathogen B. gingivalis in order to provide the tools for understanding the interactions which occur between T cells and this gram-negative bacterium. The rat was used in this study since it has been a useful animal model for investigations on the immunology and microbiology of oral inflammatory conditions associated with B. gingivalis infection (32). Therefore, splenic T cells from female Fischer rats immunized with B. gingivalis WC antigen were used to derive clones. These purified T cells were Slg<sup>+</sup> OX19<sup>+</sup>, with CD4<sup>+</sup> Th cells, and represented approximately 50% of the bulk spleen cell population. The CD4<sup>+</sup> cells were further divided based on the presence or absence of the high-molecular-weight form of the L-CA recognized by the MAb OX22. The L-CA is expressed on the surface of most hematopoietic cells, and although first found in the rat (8), it has also been recognized in other species, such as mice and humans (6, 34). Expression of this marker seems to be related to cell lineage and/or differentiation stage (26, 33). Studies on rat Th cells have shown that approximately two-thirds of the CD4<sup>+</sup> cells in the thoracic duct lymph are recognized by the MAb OX22 and thus express the L-CA determinant (29, 38). In our investigations, however, the splenic CD4<sup>+</sup> Th cells were divided into two relatively equal populations of OX22<sup>+</sup> and OX22<sup>+</sup> cells. This difference in the proportion of CD4<sup>+</sup> cells expressing the L-CA may prove to be useful in establishing to what extent the OX22 marker can be used to delineate Th cell subsets.
To generate the T-cell clones described in this report, the purified total splenic T-cell population (OX19⁺) from immunized rats was incubated with irradiated spleen cells and *B. gingivalis* at equivalent numbers, a procedure which has been useful in generating rat T-cell clones with specificity to various microbial WC (unpublished data). The cloned T cells required irradiated spleen cells and stimulation with specific antigen for growth. The antigen specificity of these T-cell clones was further confirmed by the demonstration of proliferative responses by the T cells to *B. gingivalis* antigen (Table 1) but not to other microbial antigens. Phenotypic analysis of the T-cell clones showed that they were OX19⁺ W3/25⁺ OX8⁻ and therefore a subset of CD4⁺ Th cells. Furthermore, these cells lacked the L-CA recognized by the OX22 MAb.

Studies by Mason and co-workers (1, 23, 24) have shown that phenotypic differences in the expression of the OX22 marker on rat T cells are also reflected by functional differences. CD4⁺ OX22⁺ T cells are more responsive to T-cell mitogens and produce higher levels of IL-2 than CD4⁺ OX22⁻ cells. Others (9) have reported similar results with rat T-cell clones, i.e., that CD4⁺ OX22⁻ cells are producers of low levels of IL-2 and provide B-cell help for secondary immune responses. Some functional characteristics of our T-cell clones were analogous to those described above, e.g., we have shown the production of low levels of IL-2 by our CD4⁺ OX22⁻ T-cell clones (Fig. 3). In our studies, we used the HT-2AB cells to assess IL-2 activity in SN from the T-cell clones. These indicator cells are only responsive to IL-2, although they may be less responsive to this interleu-

kin than CTLL cells, as has been reported for HT-2b cells, which also are only responsive to IL-2 (9). Thus, the proliferative response seen with HT-2AB cells incubated with SN from our T-cell clones indicated the presence of IL-2. Furthermore, the addition of anti-IL-2 antibody to similar cultures abrogated the proliferative responses (data not shown). The low level of IL-2 produced by CD4⁺ OX22⁻ cells which has been reported by others (9) and in this investigation may indeed be an inherent characteristic of this subset or it may depend upon the cell line used for the detection of this lymphokine (9). In this study, we also showed the expression of the IL-2 receptor on the cloned Th cells by FACS (Fig. 2). Whether the amount of IL-2 detected in our assays reflected quantitatively the expression of the IL-2 receptor we do not know. However, it is known (28, 36) that expression of such a receptor occurs upon stimulation of the cells by antigen or mitogen and that its presence is accompanied by cell proliferation.

The *B. gingivalis*-specific T-cell clones described in this study produced IFN-γ and some IL-2. Based on the production of these two lymphokines, it would appear that the Th cell clones generated are similar to the Th1 characterized cells in the murine system. Recent studies by Gajewski et al. (10) have shown that SN containing IL-2, IL-4, and IFN-γ preferentially support the growth of murine Th1 cell clones, which could help explain the derivation of the present rat T-cell clones. Nevertheless, the low level of detectable IL-2 in the SN of the cloned T cells may suggest still another subset of Th cells, which, however, may be unique to the rat system or to the culture conditions used for their generation. Further studies are required to delineate Th cell subsets in the rat based on phenotype, function, and lymphokine
production in order to understand their role in host responses to antigenic challenge.

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LITERATURE CITED


36. Waldmann, T. A. 1986. The structure, function, and expression

Infect. Immun.

