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Humoral Response to *Porphyromonas* (*Bacteroides*) *gingivalis* in Rats: Time Course and T-Cell Dependence

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In this study, we describe the time course and T-cell dependence of the serum antibody response to the periodontopathogen *Porphyromonas* (*Bacteroides*) *gingivalis* in an experimental rat model. Normal Fischer rats were challenged by a local injection of *P. gingivalis* (2×10^8 bacteria) into gingival tissue or by the administration of a similar number of bacteria by the intravenous (i.v.) route on days 0, 2, and 4. Serum antibody activity was detected within 1 week and peaked at 8 weeks after gingival challenge. A similar but lower response was seen for rats challenged by the i.v. route. The response in both groups of rats was mainly of the immunoglobulin G (IgG) isotype; some IgM but no IgA antibody activity was detected. Analysis of the IgG subclass revealed mainly IgG2c in animals challenged locally in the gingiva with *P. gingivalis*, whereas IgG2b predominated in rats challenged by the i.v. route. The importance of T cells in the response was established by demonstrating the absence of serum IgG antibodies in nude rats after a local challenge of gingival tissue with *P. gingivalis*. Nude rats given purified splenic T cells from normal rats immunized systemically with *P. gingivalis* prior to a local gingival challenge showed a rapid appearance of serum antibody activity that peaked between 4 and 6 weeks. This initial peak occurred 2 to 4 weeks earlier than that seen in normal animals. Fluorescence-activated cell sorter analysis of splenic lymphoid cells from these nude rats revealed a helper T-cell population. The levels of serum IgG antibodies in nude rats given nonimmune T cells rose slowly, and the antibodies were mainly of the IgG2a and IgG2b subclasses. Nude rats given immune T cells showed a rapid increase primarily in IgG2b antibody levels following a local gingival challenge. These findings suggest that the immune helper T-cells contributed to the rapid development of the response to *P. gingivalis*. Furthermore, it is likely that the IgG subclass response to *P. gingivalis* in these nude rats was related to the splenic origin of the T cells used for adoptive transfer.

Periodontitis is a chronic inflammatory disease in which the presence of a characteristic microflora has been associated with the etiology (3, 17). Among the specific oral bacteria, gram-negative, black-pigmented *Porphyromonas* (*Bacteroides*) *gingivalis* (29) has been implicated in the pathogenesis of this disease (13, 17, 30). It is thought that the interactions between the components of the infecting microorganisms and the cellular and humoral elements of the host immune system are important in the disease process. Evidence for this idea has been obtained with humans (9, 27), nonhuman primates (13), and experimental rats (35; reviewed in reference 16).

In general, the interactions that take place between *P. gingivalis* and the host are still unclear. However, studies of humoral responses in humans have reported the presence of antibodies to *P. gingivalis* in serum samples and have correlated this activity with the detection of this bacterium in gingival tissue and with disease (6, 7, 10, 22–25, 36). These results suggest that the antibodies are synthesized locally in the gingiva, although this point is still controversial (reviewed in reference 5). Furthermore, normal gingival tissue in humans (2, 28; reviewed in reference 27) and rats (34, 35) is characterized by a predominance of T lymphocytes. These T cells and the cytokines that they produce (e.g., gamma interferon, interleukin-2, and interleukin-4) could regulate the nature of the antibody response induced after a microbial challenge (reviewed in reference 21).

It is known that the nature of an antigen, the route of challenge, and the participating host cells are among the factors involved in determining different immune responses. *P. gingivalis* possesses a diversity of antigens that can be classified as either T-cell dependent or T-cell independent (reviewed in references 5 and 12), suggesting that the response(s) induced is contingent on which microbial component(s) interacts with the host immune system.

Although *P. gingivalis* is a periodontopathogen, the experiments reported here were not aimed at assessing the disease process itself but were intended to assess the humoral immune responses that can occur after a *P. gingivalis* challenge. In the present study, we investigated, using rats, the time course of the development of the serum antibody response to *P. gingivalis* after an initial challenge of gingival tissue with this bacterium. The response pattern, including the isotype and immunoglobulin G (IgG) subclass, obtained with these animals and the one obtained with rats injected with a similar dose of *P. gingivalis* whole cells (WC) by the intravenous (i.v.) route were determined. We also investigated the importance of T cells in the induction of a serum antibody response to *P. gingivalis* after a local challenge of gingival tissue with this bacterium and determined whether the splenic origin of the T cells and the presence of antigen-primed T cells affected the time course, isotype, and IgG subclass of the antibody response.

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MATERIALS AND METHODS

Animals. The inbred female Fischer rats [CDF (F-344) CrlBR; originally obtained from Charles River Breeding Laboratories, Wilmington, Mass.] and nude Fischer rats used in the experiments were derived from breeding colonies maintained at the University of Alabama at Birmingham Gnotobiotic Rat Facility. All rats were kept in horizontal laminar-flow hoods and provided food and water ad libitum. All animal experiments performed in this study were approved by the University of Alabama at Birmingham Animal Resources Advisory Committee.

Bacterial preparation. *P. gingivalis* ATCC 33277 was grown at 37°C under anaerobic conditions as described elsewhere (32, 33). *P. gingivalis* used for the local challenge of gingival tissue was grown on enriched Trypticase soy agar plates (33) and harvested immediately prior to use (see below). For use in an enzyme-linked immunosorbent assay (ELISA) and as an immunogen, killed WC of *P. gingivalis* were prepared as previously described (15). In brief, freshly harvested bacteria were suspended in sterile phosphate-buffered saline (PBS; 0.01 M, pH 7.4) containing 0.02% sodium azide and maintained at 4°C until used.

Experimental design. Groups of normal or nude rats or nude animals given immune or control splenic T cells (described below) were anesthetized with Ketalar (100 mg of ketamine plus 1.5 mg of xylazine per ml; 0.1 ml/100 g of body weight), and a suspension of freshly harvested *P. gingivalis* in sterile PBS (approximately 10^9 bacteria per ml) was injected (0.2 ml per rat) into multiple sites in the gingiva of the lower jaw. Additional groups of normal rats were challenged with *P. gingivalis* WC (2×10^8) by the i.v. route. All animals were challenged on days 0, 2, and 4. Individual rats were bled every other week from the retro-orbital plexus while under Fluorothane (1.3% halothane) sedation. The blood was allowed to clot at 4°C and, following centrifugation, the serum was collected and stored at −20°C until assayed for antibody activity by the ELISA (described below).

Generation of immune splenic T cells and adoptive transfer to nude rats. For the generation of immune T cells (15), normal female rats (8 to 12 weeks of age) were given a single intraperitoneal injection (0.5 ml) of *P. gingivalis* ATCC 33277 WC in complete Freund adjuvant (3×10^8 cells per ml). About 8 weeks later, the rats were given an i.v. injection (0.25 ml) of *P. gingivalis* WC in pyrogen-free saline (5×10^8 cells per ml); 3 days later, the rats were sacrificed for the isolation and purification of splenic T lymphocytes (described below). This population of cells is hereafter termed immune T cells. Nude Fischer rats (12 to 16 weeks of age) were given immune T cells (2×10^7 per rat) by i.v. injection (0.5 ml). As controls, nude rats were injected with splenic T cells (2×10^7 per rat) derived from nonimmunized normal rats or with PBS only. Rats were challenged locally in the gingival tissue with *P. gingivalis* within 18 to 24 h after adoptive transfer of cells.

Isolation and phenotypic characterization of splenic lymphoid cells. The procedures used for the isolation and purification of splenic lymphoid cell populations and the monoclonal antibodies (MAbs) used for their characterization were the same as those described previously (15). In brief, rats were sacrificed 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, Grand Island, N.Y.). Following lysis of erythrocytes with ammonium chloride buffer, the cells were passed through a Sephadex G-10 (Pharmacia, Piscataway, N.J.) column equilibrated with warm (37°C) RPMI 1640 supplemented with 5% fetal calf serum. For the adoptive transfer studies, the eluted cell population was depleted of B cells by being panned on plastic petri plates (15 by 100 mm; Falcon Labware, Oxnard, Calif.) coated with rabbit IgG anti-rat $F(ab')_2$ (8, 20). After incubation for 90 min at 4°C, the nonadherent cell population was collected, washed twice with RPMI 1640, and resuspended in RPMI 1640 to the appropriate cell concentration for injection. The resulting cell population was determined by trypan blue exclusion to be >98% viable.

Splenic lymphoid cell populations were characterized (15) by use of mouse MAbs OX19, specific for rat T lymphocytes (CD5); W3/25, specific for the rat T helper/inducer (Th) subpopulation (CD4); OX8, specific for the T suppressor/T cytotoxic populations (CD8) (cell lines were kindly provided by Allan F. Williams, Medical Research Council, Cellular Immunology Unit, Oxford University, Oxford, England); and MAR 18.5, specific for the rat immunoglobulin kappa chain (18). Cell populations were phenotypically characterized following incubation of aliquots of cell preparations with the appropriate fluorescein isothiocyanate (FITC)- or biotin-conjugated MAb and analysis with a FACStar (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) fluorescence-activated cell sorter (FACS). The avidin-phycoerythrin used with the biotinylated reagents was purchased from Southern Biotechnology Associates, Inc., Birmingham, Ala.

FACS analysis of the Sephadex G-10-passed and anti-rat $F(ab')_2$-nonadherent cell populations from *P. gingivalis*-immunized rats or control rats showed that they consisted of $CD5^+$ T cells (OX19 positive) and <3% surface immunoglobulin-positive cells, as established with MAb MAR 18.5 (Fig. 1). Approximately equal proportions of $CD4^+$ (~44%) and $CD8^+$ (~48%) cells were present in the cell populations, as determined with MAbs W3/25 and OX8, respectively.

ELISA. The ELISA used for assessing antibody activity against *P. gingivalis* was similar to that described previously (4, 14, 19). In brief, individual wells of flat-bottomed 96-well plates were coated with *P. gingivalis* WC (100 μl; 5×10^8/ml) in sodium carbonate buffer (pH 9.6) by overnight incubation (37°C) and then blocked with fetal calf serum (5% in PBS containing 0.05% Tween 20). Four twofold dilutions of serum samples or six twofold dilutions of a serum standard (described below) were added in quadruplicate to individual wells, and the plates were incubated for 2 h at room temperature. Biotin-conjugated anti-rat immunoglobulin, IgM, and IgG (Southern Biotechnology Associates), IgG1, IgG2a, IgG2b, and IgG2c (The Binding Site, Inc., San Diego, Calif.), or IgA (Zymed Laboratories, Inc., San Francisco, Calif.) was added to appropriate wells, and then streptavidin-alkaline phosphatase (0.4 mg/ml; Southern Biotechnology Associates) was added. After the addition of the phosphatase substrate (Sigma 104) in diethanolamine buffer, color development was recorded at 405 nm with a Titertek Multiskan reader (Flow Laboratories, Inc., McLean, Va.). A four-parameter log-logit program (Assayzap; Elsevier-Biosoft, Cambridge, United Kingdom) was used to construct

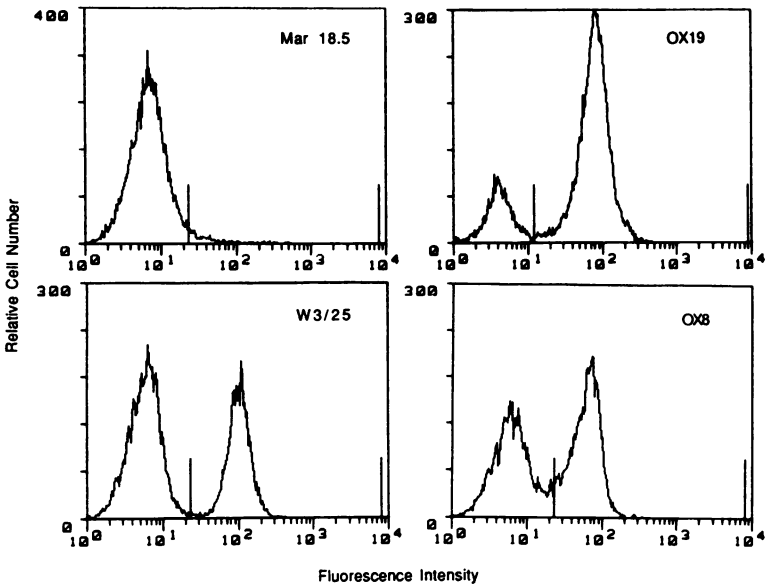

FIG. 1. Analysis by flow cytometry of cell surface markers on rat splenic lymphoid cell populations used for adoptive transfer into nude rats. Rat spleen cells were initially passed through a Sephadex G-10 column and, following panning on anti-rat $F(ab')_2$-coated plates, the nonadherent cell populations were collected. Aliquots (2×10^6 cells) of the cell preparations were stained with FITC-labeled anti-rat reagent or biotinylated reagent and avidin-phycoerythrin (see Materials and Methods). Profiles are representative of four different analyses of similarly prepared cell populations.

reference curves for each ELISA plate from optical density readings for appropriate dilutions of a standard serum pool (see below) assigned ELISA units (EU). Optical density readings for sample dilutions that were on the linear portion of the curve were converted to EU values and multiplied by the dilution factor to determine the EU of antibody activity per milliliter for each sample.

The serum standard used in the ELISA was a pool of serum obtained from a group of rats given weekly i.v. injections for 6 weeks of increasing numbers of killed *P. gingivalis* WC (1×10^7 to 5×10^8) and bled 1 week after the last immunization. Aliquots of the standard serum pool were stored frozen (−20°C) until used.

Statistics. Data were analyzed by the unpaired Student's *t* test, and differences were considered statistically significant at $P \leq 0.05$.

RESULTS

Humoral responses to *P. gingivalis* in normal rats. To study humoral responses to *P. gingivalis*, we challenged groups of normal Fischer rats with *P. gingivalis* ATCC 33277 either by the natural route of infection, i.e., in the gingival tissue, or by the i.v. route. Individual serum samples were collected every other week and assessed for the level, isotype, and IgG subclass of the antibody responses over a 3- to 4-month period. The level of total serum anti-*P. gingivalis* activity detected was higher at weeks 4, 6, 8, and 14 in rats after a local challenge of the gingiva than in rats given a similar dose of the bacterium by the i.v. route (Fig. 2). The peak antibody activity in both groups occurred at 8 weeks. In the former group (local challenge), a subsequent increase in the antibody level was noted after a decline.

The responses seen in these two groups of animals were mainly of the IgG isotype (Table 1) and followed a pattern similar to that seen in Fig. 2. By 4 weeks after administration of *P. gingivalis*, the IgG response in the locally challenged group was significantly higher than that in the i.v.-challenged group. This difference in IgG antibody levels in the two groups of rats was seen throughout the following 10 weeks. Some IgM but no IgA antibody activity was detected in both groups of animals.

Examination of the IgG subclass antibody responses in

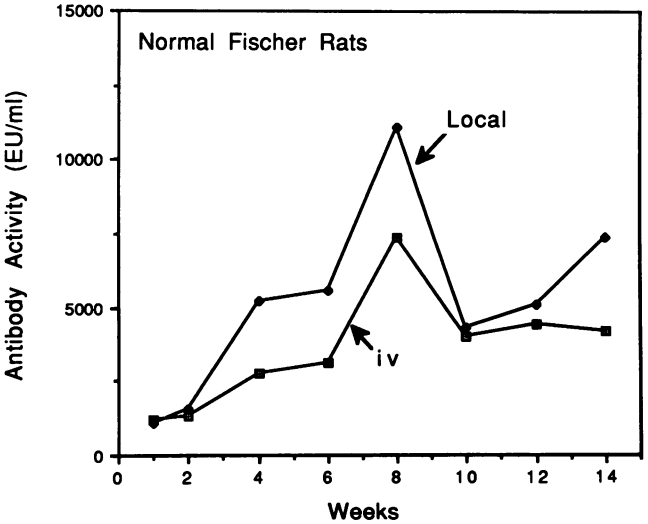

FIG. 2. Time course of the serum antibody responses to *P. gingivalis* in normal Fischer rats. Groups of six rats were challenged with *P. gingivalis* ATCC 33277 by injection of the bacteria into the gingival tissue (local route) or via the i.v. route. Serum samples were collected every other week from individual animals and assessed for total antibody activity by the ELISA (see Materials and Methods). Values are the mean EU per milliliter in six individual samples per group, with a standard error of the mean of less than 20%, and are representative of two separate experiments.

TABLE 1. Serum IgM and IgG antibody responses in normal rats after a challenge with *P. gingivalis* either by the local route (in the gingival tissue) or by the i.v. route[a]

Wk	Activity (EU/ml)[b] of the indicated antibody in rats challenged by the following route:			
	Local		i.v.	
	IgM	IgG	IgM	IgG
1	532 ± 65	225 ± 41	488 ± 58	305 ± 55
2	377 ± 59	375 ± 45	467 ± 61	595 ± 71
4	237 ± 39	2,080 ± 212[c]	307 ± 46	1,059 ± 125
6	153 ± 28	3,544 ± 362[c]	29 ± 9	1,565 ± 201
8	144 ± 23	7,807 ± 810[c]	25 ± 8	5,414 ± 510
10	202 ± 28	5,203 ± 697[c]	22 ± 8	3,608 ± 420
12	401 ± 62	6,872 ± 754[c]	409 ± 54	2,290 ± 290
14	246 ± 42	7,351 ± 808[c]	260 ± 41	2,582 ± 310

[a] Groups of normal rats were challenged with *P. gingivalis* ATCC 33277 by the local or the i.v. route and bled every other week for 14 weeks. Serum was assayed for IgM, IgA, and IgG antibody activities by the ELISA (see Materials and Methods).

[b] Values are the mean ± standard error of the mean for serum from six rats per group and are representative of two separate experiments. No IgA antibody activity was detected.

[c] Values significantly different between groups at $P < 0.05$.

rats challenged locally in the gingiva with *P. gingivalis* showed that the major subclass was IgG2c (Fig. 3A). Antibody activities of the other IgG subclasses were also detected, and their levels tended to increase with time following challenge. On the other hand, rats challenged with *P. gingivalis* WC by the i.v. route showed primarily an IgG2b response (Fig. 3B), which peaked at 8 weeks. These results indicate that the IgG subclass of antibodies elicited to *P. gingivalis* differs depending on the route of challenge. However, differences in the preparations of *P. gingivalis* used for gingival (live bacteria) and i.v. (killed WC) administration may also have accounted for the observed host responses.

Effect of immune T cells on humoral responses to *P. gingivalis*. To assess the role of T cells in regulating the serum antibody responses induced after a local gingival challenge with *P. gingivalis*, we gave groups of nude Fischer rats T cells isolated from the spleens of *P. gingivalis*-immunized normal Fischer rats (see Materials and Methods). After adoptive transfer of the T cells into nude rats, the animals were locally challenged in the gingiva with *P. gingivalis* and then individual serum samples were collected every other week for 3 to 4 months and assessed for the level, isotype, and IgG subclass of the antibody responses. A rapidly appearing serum antibody response occurred in these rats (Fig. 4). The response increased quickly and peaked within 4 to 6 weeks. Following a decline, an increase in antibody activity was seen. This response pattern differed from that seen for similarly treated normal Fischer rats (Fig. 2), in which the response increased more slowly and peaked at 8 weeks. The observed significantly lower response in locally challenged nude rats that received no T cells (Fig. 4) demonstrated the importance of T cells in serum antibody responses to *P. gingivalis*. Furthermore, these results suggest that the rapid appearance of antibody activity in the nude rats was due to the adoptively transferred *P. gingivalis*-sensitized T cells.

FACS analysis of splenic lymphoid cells from nude rats given T cells showed that the population consisted of surface immunoglobulin-positive B cells (~50%) and W3/25-positive Th cells (~25%) (Fig. 5, left panels), whereas splenic lymphoid cells from nude rats given no T cells consisted primarily of surface-immunoglobulin-positive B cells (~70%) and no W3/25-positive Th cells (Fig. 5, right panels). No difference in the OX8-positive cell population was seen in the two groups (data not shown). These results suggest that the Th cell population accounted for the responses observed in these animals after a local gingival challenge with *P. gingivalis*.

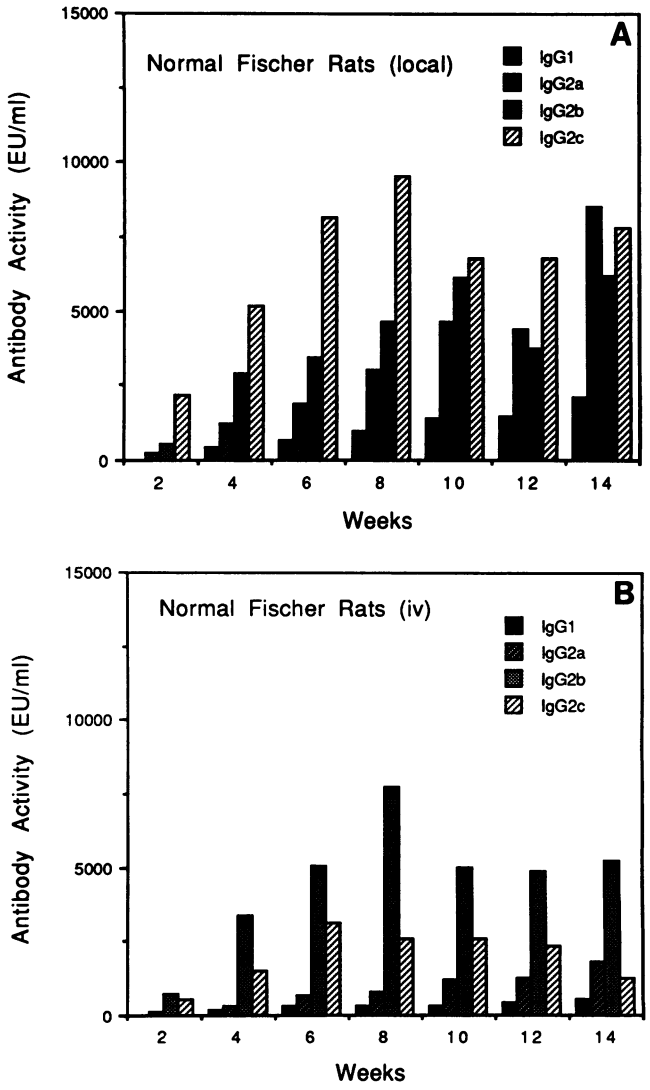

FIG. 3. Time course of the serum IgG subclass antibody responses to *P. gingivalis* in normal Fischer rats. Groups of six rats were challenged with *P. gingivalis* ATCC 33277 by injection of the bacteria into the gingival tissue (local route; A) or via the i.v. route (B). Serum samples were collected every other week from individual animals and assessed for IgG subclass antibody activity by the ELISA (see Materials and Methods). Values are the mean EU per milliliter in six individual samples per group, with a standard error of the mean of less than 20%, and are representative of two separate experiments.

We next evaluated the isotypes and levels of serum antibodies in nude rats and in nude rats given nonimmune or immune T cells (Table 2). The IgG response in rats given nonimmune T cells rose more slowly and reached a significantly lower level than that in rats given immune T cells.

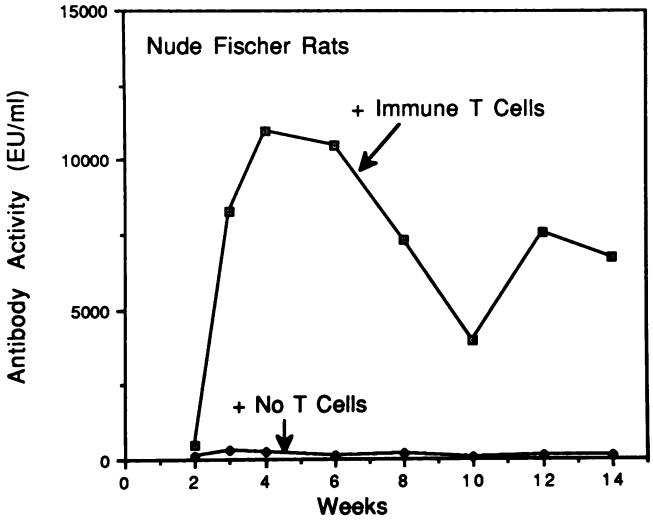

FIG. 4. Time course of the serum antibody responses to *P. gingivalis* in nude rats (+ No T cells) or nude rats given i.v. purified splenic T cells from normal rats immunized with *P. gingivalis* (+ Immune T cells). Six rats per group were challenged with freshly harvested *P. gingivalis* ATCC 33277 by injection of the bacteria into the gingival tissue. Serum samples were collected every other week from individual animals and assessed for total antibody activity by the ELISA (see Materials and Methods). Values are the mean EU per milliliter in six individual samples per group, with a standard error of the mean of less than 20%, and are representative of two separate experiments.

These results suggest that the difference in the response patterns was due to the presence of antigen-sensitized Th cells in the latter group. In the group that did not receive any T lymphocytes, no IgG antibody activity was detected. In all groups, some IgM but no IgA antibody activity was noted.

TABLE 2. Kinetics of the serum antibody responses in nude rats given immune or nonimmune T cells and challenged with *P. gingivalis*[a]

Wk	Activity (EU/ml)[b] of the indicated antibody in nude rats given the following T cells:				
	None (IgM)[c]	Nonimmune		Immune	
		IgM	IgG	IgM	IgG
1	350 ± 45	375 ± 49	210 ± 39[d]	310 ± 52	999 ± 89
2	420 ± 79	389 ± 53	438 ± 77[d]	327 ± 49	2,032 ± 472
4	510 ± 87	480 ± 61	927 ± 115[d]	453 ± 61	8,745 ± 1,055
6	179 ± 22	250 ± 52	1,184 ± 181[d]	246 ± 30	7,887 ± 1,012
8	NT	564 ± 78	1,571 ± 273[d]	307 ± 38	6,691 ± 986
10	322 ± 45	610 ± 110	4,219 ± 686	450 ± 61	4,932 ± 762
12	NT	308 ± 51	4,519 ± 707[d]	422 ± 65	7,583 ± 1,125
14	NT	25 ± 7	5,115 ± 866	37 ± 8	6,994 ± 1,005

[a] Groups of nude rats or of nude rats given nonimmune or immune T cells (2×10^7) were challenged with *P. gingivalis* ATCC 33277 in the gingival tissue and bled every other week for 14 weeks. Serum was assayed for IgM, IgA, and IgG antibody activities by the ELISA (see Materials and Methods).

[b] Values are the mean ± standard error of the mean for six rats per group and are representative of two separate experiments. No IgA antibody activity was detected.

[c] No IgG antibody activity was detected (1/100 dilution of serum samples). NT, not tested.

[d] Values significantly different between groups at $P < 0.05$.

Analysis of the serum IgG subclass antibody responses in nude rats given nonimmune T cells showed IgG2a and IgG2b antibodies to be the most prevalent in the low response seen in this group (Fig. 6A). On the other hand, the responses in nude rats given immune T cells were characterized by IgG2b antibodies, the levels of which increased rapidly after a local gingival challenge with *P. gingivalis* (Fig. 6B). This response was significantly higher than the IgG2b response seen in nude rats given nonimmune T cells (Fig. 6A).

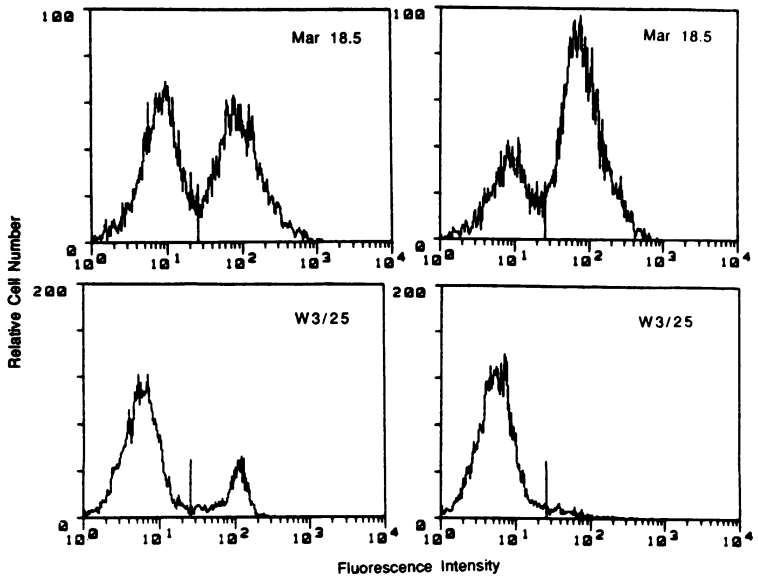

FIG. 5. FACS analysis of Sephadex G-10-passed spleen cells from experimental nude rats approximately 4 months after adoptive transfer of T cells (2×10^7 per rat) (left panels) and from control nude rats (right panels). Rat spleen cells were passed through a Sephadex G-10 column, and aliquots (2×10^6 cells) of the cell preparations were stained with FITC-labeled anti-rat reagent or biotinylated reagent and avidin-phycoerythrin (see Materials and Methods). Profiles are representative of the results obtained in analyses of cell populations in three separate experiments.

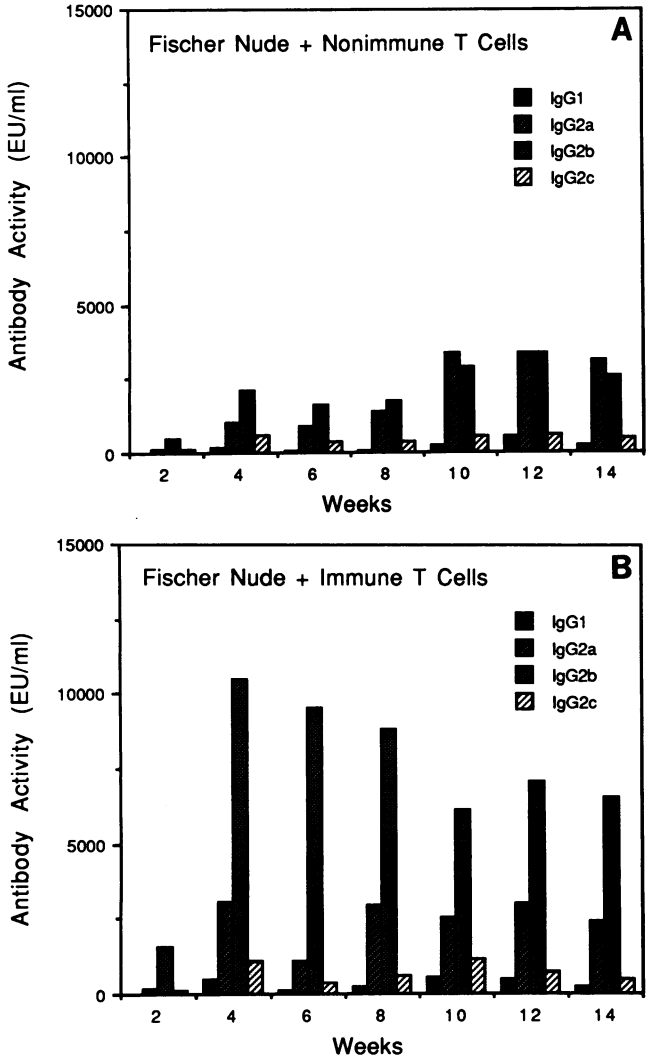

FIG. 6. Time course of the serum IgG subclass antibody responses to *P. gingivalis* in nude Fischer rats given nonimmune (A) or immune (B) T cells by adoptive transfer. Groups of six rats were given i.v. purified splenic T cells from normal rats immunized with *P. gingivalis* ATCC 33277 or from normal nonimmunized rats and then were challenged with freshly harvested *P. gingivalis* by injection of the bacteria into the gingival tissue. Serum samples were collected every other week from individual animals and assessed for IgG subclass antibody activity by the ELISA (see Materials and Methods). Values are the mean EU per milliliter in six individual samples per group, with a standard error of the mean of less than 20%, and are representative of two separate experiments.

DISCUSSION

The pathogen *P. gingivalis* is a gram-negative, black-pigmented bacterium that has been implicated in the etiology of periodontitis (3, 13, 17, 30). The pathologic process in general seems to be determined by the interactions between the infecting microorganisms of the bacterial plaque and the host defense mechanisms (9, 13, 27, 35). The present investigation was undertaken to shed some light on the humoral immune responses that occur upon challenge of rats with one of the microbial components of plaque, namely, *P. gingivalis*. In this paper, we present results on the level, isotype, and IgG subclass of antibody activity, as determined by an ELISA, in serum samples collected from individual animals over a period of approximately 4 months.

In the present study, a gingival challenge of normal Fischer rats with *P. gingivalis* resulted in a response that was mainly of the IgG isotype (Table 1). Upon further analysis, it was determined that the predominant subclass of the response was IgG2c, although the levels of the other subclasses increased with time (Fig. 3). On the other hand, an i.v. challenge of rats with *P. gingivalis* resulted in a lower, more restricted subclass response, which consisted primarily of IgG2b antibodies. The response patterns seen in these groups could reflect differences in the route of challenge, i.e., local gingival versus i.v. It is also possible that they relate to the antigen form, i.e., live (local) versus killed (i.v.). Furthermore, antigens can influence immune responses depending on their T-cell dependency. *P. gingivalis* expresses a variety of antigens capable of inducing humoral responses (5, 12), and our results with nude rats demonstrated that unless T cells were present, no serum IgG antibody was detected after a local gingival challenge with this bacterium (Table 2). It was also of interest that nude rats given immune splenic T cells showed predominantly an IgG2b subclass response after a local gingival challenge with *P. gingivalis* (Fig. 6), whereas in normal rats the dominant subclass was IgG2c (Fig. 3). We propose that the former response reflected regulation by antigen-primed splenic T cells, since it resembled that obtained in normal rats given *P. gingivalis* by the i.v. route (Fig. 3B) and not that obtained in normal animals following a local gingival challenge (Fig. 3A). At this point in time, we do not know how much local gingival factors influence the serum antibody response after a microbial challenge of gingival tissue. More work is necessary to establish the nature of T cells that may account for the different responses.

Various investigations have established that the IgG subclass distribution of humoral immune responses to microbial antigens is regulated by cytokines produced by subsets of Th cells (11, 21, 31). Extensive studies with the murine system have shown that interleukin-4 produced by Th2 cells supports IgG1 responses, whereas gamma interferon produced by Th1 cells augments IgG2a antibody production (11, 21, 31). The correlate of the latter subclass in rats is IgG2b (1), the predominant antibodies observed in normal rats injected with *P. gingivalis* by the i.v. route or in nude rats given immune splenic T cells and challenged in the gingival tissue. We have reported that *P. gingivalis*-specific Th cell clones derived from the spleens of rats systemically immunized with this bacterium produce interleukin-2 and gamma interferon in vitro (14, 15). In the present study, it was shown that Th cells contributed to the in vivo response in nude rats given splenic immune T cells. Therefore, we could infer that gamma interferon produced by a subset of Th cells was another factor that influenced the subclass response to *P. gingivalis*. Further studies are needed to support this possibility.

In summary, in the present paper, we describe the development of a serum antibody response after a challenge of gingival tissue of rats with *P. gingivalis*. We have also shown the importance of antigen-sensitized Th cells in providing help for the rapid development of a serum IgG antibody response following a local gingival challenge with *P. gingivalis*. In addition, evidence that these adoptively transferred T cells influence the subclass of this response has been presented. Ongoing investigations in our laboratory are determining the specificity of antibodies produced in *P. gingivalis*-challenged rats, the characteristics of T lympho-

cytes and cytokines involved in regulating immune responses to this microorganism, and the importance of these cellular and humoral elements in the pathogenesis of disease.

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