Vaccine-Specific T Cells in Human Peripheral Blood after Oral Immunization with an Inactivated Enterotoxigenic Escherichia coli Vaccine

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Received 11 January 1993/Returned for modification 1 March 1993/Accepted 29 December 1993

We have examined whether oral immunization of adult Swedish volunteers with a prototype enterotoxigenic Escherichia coli vaccine would induce antigen-specific T-cell responses in blood. Volunteers were given one to three doses of the whole-cell component of the vaccine, which consisted of formalin-inactivated bacteria expressing the fimbrial colonization factor antigens I and II. Following immunization, in vitro stimulation of blood mononuclear cells with the colonization factor antigens resulted in modest proliferative responses which were accounted for mainly by CD4+ T cells and, to a lesser extent, by CD8+ T cells. A main finding of this study was that a majority of the orally immunized volunteers had circulating T cells capable of producing large quantities of gamma interferon following in vitro exposure to either of the colonization factor antigens. No interleukin 2 production could be detected in the cell cultures. These results suggest that oral immunization of humans induces the migration of specific mucosal T lymphocytes from the intestine into peripheral blood.

It is generally held that antigen administered in the gastrointestinal tract can induce activation of intestinal lymphocytes, a fraction of which may migrate via the mesenteric lymph nodes and the thoracic duct to the circulation before returning to the site of antigen encounter and to other mucosal tissues such as salivary and mammary glands. This migratory behavior has been extensively documented for mucosal B cells in both animal and human systems (7, 14, 21, 33). In regard to the migration of T cells, it has been shown in adoptive cell transfer experiments that T blasts from mesenteric lymph nodes under certain conditions preferentially repopulate the gut upon transfer to syngeneic recipients (11, 22). Furthermore, it has been demonstrated that antigen-specific T-helper cells arising in the Peyer's patches after intra-Peyer's patch immunization subsequently appeared in the thoracic duct, the intestinal epithelium, and the lamina propria of the gut (8). The aim of the present study was to investigate whether intestinal T cells, which play an essential role in controlling B-cell proliferation and differentiation, would display similar migratory properties in humans.

We have recently developed a prototype vaccine against enterotoxigenic Escherichia coli (ETEC) (29, 30), which is the most common enteropathogen in humans (4). ETEC colonizes the small intestine by means of fimbrial colonization factor antigens (CFAs) and gives rise to diarrhea by the secretion of one or two enterotoxins. In previous studies, we have shown that oral immunization of human volunteers with this prototype ETEC vaccine evoked a specific mucosal immunoglobulin A (IgA) response in intestinal lavage fluid (1) and the transient appearance of CFA-specific IgA- and IgM-secreting cells in the circulation (34). On the basis of these results, we examined whether oral immunization with the ETEC vaccine would give rise to vaccine-specific T cells in human peripheral blood.

(These results were presented in part at the 7th International Congress of Mucosal Immunology, Prague, Czechoslovakia, August 1992.)

MATERIALS AND METHODS

Subjects and immunizations. Twenty-one healthy adult individuals, 13 men and 8 women, that were 20 to 52 years old volunteered to participate in this study (which was approved by the Ethical Committee of the University of Göteborg). Four of the volunteers were given one dose, 14 were given two doses, and three were given three doses of the whole-cell component of a prototype ETEC vaccine that was produced by the Swedish National Bacteriological Laboratory. Each dose of vaccine, which consisted of 10^11 formalin-killed ETEC bacteria expressing CFA/I and CFA/II (comprising the coli surface components CS1, CS2, and CS3), was mixed with 150 ml of a sodium-bicarbonate-citric acid buffer (ACO Pharmachemicals, Stockholm, Sweden) and was given as a drink with 2-week intervals between immunizations (with the exception of four volunteers who were immunized with a 3-day interval). Volunteers fasted for 2 h before and 1 h after immunization. Ten to 50 ml of heparinized blood was collected from all volunteers before and at weekly intervals following each immunization.

Purified antigens. Purified CFA/I was prepared from a flagellum-deficient mutant strain of H10407 (O78K80:H11, heat-stable toxin/heat-labile toxin) by homogenization with a Waring blender followed by ammonium sulfate fractionation and negative DEAE Sephadex column chromatography (9). Purified CFA/II (CSI plus CS3) was prepared from strain E1392-75 (O6K15:H16, heat-stable toxin/heat-labile toxin) by homogenization followed either by salt precipitation and column chromatography (18) or by cesium chloride gradient ultracentrifugation (19). The purity of the CFA/I and CFA/II preparations obtained was ascertained by immunoblot analyses showing single bands corresponding to the different CFA subunit proteins when antisera against whole fimbriated bacteria were used.

Lymphocyte proliferation assays. Peripheral blood mononuclear cells (PBMC) were isolated by standard density gradient
centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells were seeded into round-bottom 96-well culture plates (Nunc, Roskilde, Denmark) at two different concentrations (4 × 10^5 and 2 × 10^5/ml) in a final volume of 0.2 ml of Iscove's medium supplemented with 10% fetal calf serum (Biological Industries), and 0.1 mg of gentamicin (Essex, Lakemedel AB, Sweden), 3 μg of L-glutamine ( GibCO, Edinburgh, United Kingdom), 1 mg of folic acid (E. Merck, Darmstadt, Germany), 12 mg of L-arginine monohydrochloride (Sigma Chemical Co., St. Louis, Mo.), 3.6 mg of asparagine monohydrate (Sigma), and 0.39 mg of 2-mercaptoethanol (Carl Roth KG, Karlsruhe, Germany) per ml. Purified CFAs were added to the wells in three different dilutions (CFA/II at the final concentrations of 22, 7, and 2 μg/ml and CFA/II at 9, 3, and 1 μg/ml). Parallel sets of wells containing PBMC exposed to medium alone and to phytohemagglutinin (Wellcome Diagnostics, Dartford, United Kingdom) were included in all experiments as internal negative and positive controls, respectively. The cultures were maintained for 4 days at 37°C in a humidified atmosphere supplemented with 7.5% CO2. Twenty-four and 48 h after initiation of the cultures, aliquots of supernatants were collected and frozen at −18°C until being assayed for interleukin 2 (IL-2) and gamma interferon (IFN-γ) contents, respectively. After the completion of the 4-day culture period, 1 μCi of [3H]-thymidine (Radiochemical Centre, Amersham, United Kingdom) was added to each well. Sixteen hours later, cells were harvested in an automated filter cell harvester (Inotech, Basel, Switzerland), and incorporation of radioactive thymidine into newly synthesized DNA was measured with an argon-activated-β-scintillation counter (Inotech). Proliferative responses were expressed as stimulation indices (SI), defined as the ratio of [3H]-thymidine incorporated by optimal numbers of PBMC (determined for each individual) cultured in the presence of optimal amounts of CFA/II or CFA/II (determined for each individual) to that incorporated by cells similarly cultured but in the absence of antigen. Triplicate cultures were evaluated, and median values were chosen instead of means, because there were large variations (up to 40%) between replicate cultures. SI values ≥2 were considered positive.

CD4+ and CD8+ T cells were isolated from peripheral blood by the following procedure. Initially, CD2+ cells were obtained by rosetting PBMC with 2-amoethyi isothiouro- ium bromide-treated sheep erythrocytes (16) and subjecting them to density gradient centrifugation on Ficoll-Paque. The rosetted cells were removed from the erythrocytes by hypo-osmotic shock, yielding a CD2+ T-cell enriched fraction (>95% as judged by flow cytometric analyses with a fluorescence-activated cell sorter [FACS]). Thereafter, 3 × 10^6 CD2+ T cells were mixed with 30 × 10^6 magnetic microspheres coated with monoclonal antibodies specific for human CD8 or CD4 (Dynal AS, Oslo, Norway), pelleted by low-speed centrifugation (150 × g, 5 min), and then incubated for 45 min at 4°C. The pellets were carefully resuspended, and a magnetic field was applied to the side of the vial; nonrosetted cells were collected by aspiration. FACS analyses revealed that this immunomagnetic depletion procedure removed ≥95% of the CD4+ or CD8+ T cells.

Plastic-adherent cells (nominal monocytes) were employed as a source of accessory cells. These were obtained by incubating 15 × 10^6 nonfractionated PBMC on 9-cm-diameter polystyrene petri dishes at 37°C for 1 h, after which nonadherent cells were removed by aspiration. After two additional washes with phosphate-buffered saline (PBS), the petri dishes were filled with Iscove’s medium supplemented with 2.5% fetal calf serum and incubated overnight at 4°C to elute plastic-adherent cells.

Triplicate cultures consisting of 10^5 CD4+ (or CD8+) cells and 5 × 10^5 irradiated (2,500 rads) accessory cells were established. Cells were cultured for 4 days in the presence and absence of purified CFAs before the addition of [6-3H]thymidine. Proliferative responses were determined as above.

IL-2 assay. The IL-2 contents of cell microcultures were determined by the CTLL-2 bioassay of Gilliss et al. (10). PBMC were cultured for 24 h with CFA/I, CFA/II, phytohemagglutinin, or medium alone, and thereafter supernatants were collected, frozen at −18°C, and assayed for IL-2 activity within 4 weeks. (Under these storage conditions, IL-2 activity in supernatants from phytohemagglutinin-stimulated PBMC remained stable for at least 4 months.) IL-2 activity in supernatants was determined by extrapolation from a standard curve generated by adding known amounts of purified recombinant human IL-2 (Genzyme Corporation, Cambridge, Mass.) to CTLL-2 cells and measuring cell proliferation by the incorporation of [6-3H]thymidine, as described above.

IFN-γ assays. Numbers of IFN-γ-secreting cells and amounts of cell-free IFN-γ generated in cultures of PBMC exposed for 48 h to either of the two CFAs were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) (2) and a reverse ELISPOT assay (6), respectively. At the completion of the culture period, cells and supernatants were separated by centrifugation at 400 × g for 10 min. Supernatants from duplicate cultures were harvested and kept frozen at −20°C until being assayed by ELISA (see below). The cell pellets were resuspended with sterile PBS, washed twice, resuspended in complete culture medium, and assayed for numbers of IFN-γ-secreting cells by ELISPOT (see below). The IFN-γ-specific monoclonal antibodies used in both the ELISA and ELISPOT assay were purified by protein A affinity chromatography and were kindly provided by Gudrun Andersson (Research and Development Immunobiology, Kabi Bio- pharma, Stockholm, Sweden) (2).

(i) IFN-γ ELISA. Ninety-six-well polystyrene microplates (Flow Laboratories, Erving, Scotland) were coated with a monoclonal antibody directed against IFN-γ at a concentration of 5 μg/ml in PBS, pH 7.2. Thereafter, 75 μl of the undiluted supernatants of PBMC cultured for 48 h in the presence or absence of purified CFAs was added to the plates, and the plates were incubated overnight at 4°C. Plates were developed by the sequential addition of biotinylated monoclonal antibody (diluted to 2 μg/ml in PBS with 0.05% Tween 20) with specificity for another epitope of IFN-γ; 2 μg of Extravidin (Sigma Chemical Co.) per ml conjugated to horseradish peroxidase; and the chromogen-substrate 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)–H2O2 (Sigma). The IFN-γ contents of the samples were estimated from a standard curve, generated by the inclusion of known amounts of purified recombinant human IFN-γ (2). Variations between replicate cultures did not exceed 15%.

(ii) IFN-γ ELISPOT. Cultured cells (3.2 × 10^5 per well) were added in duplicate to nitrocellulose-bottom 96-well plates (Millititer HA; Millipore Corp., Bedford, Mass.) which had previously been coated with an anti-IFN-γ monoclonal antibody (5 μg/ml in PBS; 16 h at 4°C). Plates were incubated for 20 h at 37°C in a humid atmosphere with 7.5% CO2. Zones of solid phase-bound IFN-γ (secreted from individual cells) were revealed by the sequential addition of biotinylated mouse monoclonal antibody specific for IFN-γ, horseradish peroxidase-labelled avidin (Extravidin, Sigma Chemical Co.), and chromogen-substrate 3-amino-9-ethylcarbazole–H2O2 (Sigma) as described in detail elsewhere (6). Spots were enumerated under low magnification (×40). Data were adjusted to num-
RESULTS

CFA/I- and CFA/II-induced proliferative responses. Purified CFA/I and CFA/II were both capable of inducing in vitro proliferation of PBMC collected from volunteers orally immunized with the ETEC vaccine. Thus, cells from 10 of 13 vaccinees had a twofold or higher SI to CFA/I, and cells from 6 of 13 volunteers had a corresponding response to CFA/II (Fig. 1). The arithmetic mean preimmune SI were 1.4 for CFA/I and 1.7 for CFA/II. After immunization, SI increased on the average 2.8-fold after stimulation with CFA/I and 1.9-fold after stimulation with CFA/II; both these increases were statistically significant (Fig. 1). Radioactive thymidine incorporation ranged from 1,000 to 5,000 cpm in cultures containing PBMC exposed to medium alone (negative control) and from 1,000 to 20,000 cpm (SI = 0 to 16) in cultures of CFA-stimulated PBMC from vaccines. Incorporation of radioactive thymidine in cultures of PBMC exposed to phytohemagglutinin ranged from 50,000 to 150,000 cpm (SI = 30 to 150). Overall, CFA-induced proliferative responses were rather moderate and varied considerably between individuals.

We could also document that the proliferative responses to CFA/I and CFA/II were accounted for mainly by CD4+ T cells and, to a lesser extent, by CD8+ T cells (Fig. 2). After immunization, a twofold or higher proliferative response to CFA/I and CFA/II was observed for the CD4+ T-cell fractions isolated from four of eight and three of eight volunteers, respectively. Corresponding results for the CD8+ T-cell fraction were two of eight and three of eight for CFA/I and CFA/II, respectively. A comparison between preimmune and postimmune SI revealed statistically significant differences for the CD4 fraction stimulated by either of the CFAs and for the CD8 fraction stimulated by CFA/I (Fig. 2). However, even

FIG. 1. In vitro proliferative responses of human PBMC to ETEC vaccine components. The data are SI determined on triplicate cultures of PBMC stimulated in vitro with CFA/I or CFA/II, isolated from 13 human volunteers before and after oral ETEC vaccination. The indicated postimmune (Post) SI values were the maximal ones recorded for each individual, that is, after either one, two, or three oral ETEC immunizations. Statistical significance levels between the means of pre- and postimmune SI values were determined by the paired twailed Student's t test (with 12 degrees of freedom) after log transformation.

FIG. 2. In vitro proliferative responses of CD4+ and CD8+ T cells to ETEC vaccine components. CD4+ and CD8+ PBMC were isolated from eight volunteers before and after oral ETEC vaccination and cultured with CFA/I or CFA/II. Postimmune (Post) SI were determined 1 week after either one (four volunteers) or two immunizations (four volunteers). Statistical significance levels were determined by the paired two-tailed Student's t test (with 7 degrees of freedom) after log transformation of data.

FIG. 3. Antigen-induced IFN-γ production by PBMC after oral ETEC vaccination. The data are mean concentrations of IFN-γ in supernatants from duplicate cultures of PBMC stimulated in vitro by CFA/I or CFA/II. PBMC were obtained from 8 nonimmunized (open symbols) and 10 immunized (closed symbols) nonmatched volunteers. Postimmune (Post) values were determined 1 week after a single oral dose of the ETEC vaccine. Bars, medians. Statistical significance levels were determined by the Wilcoxon rank sum test.
Antigen-specific T cells after oral ETEC immunization

TABLE 1. IFN-γ-secreting cells in PBMC cultures exposed in vitro to CFAs

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>CFA/I</th>
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<td>Pre</td>
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*a* Pre and Post, cultures initiated before and after a single oral ETEC immunization, respectively. The data are arithmetic means for duplicate determinations.

Although the frequencies of responders were similar for the two T-cell subpopulations, responses were considerably higher in the CD4+ T-cell fraction than in the CD8+ T-cell fraction. Further, there was no apparent correlation between the magnitude of proliferative responses of either CD4+ or CD8+ T cells and the number of immunizations (data not shown).

**IL-2 responses.** The capacity of PBMC from orally immunized volunteers to produce IL-2 in response to in vitro stimulation with CFA/I and CFA/II was also examined. In no instance could we detect bioassayable amounts of IL-2 in the supernatants of in vitro-stimulated PBMC either before or following any of the oral immunizations (data not shown).

**IFN-γ responses.** We also determined the ability of PBMC isolated from oral vaccinees to produce IFN-γ in response to in vitro-added CFAs. Only low levels of IFN-γ were detected in the supernatants of PBMC isolated from eight nonimmunized individuals (arithmetic means, 54 and 24 mU/mL for CFA/I and CFA/II, respectively). As early as 1 week after a single oral immunization with the whole-cell component of the ETEC vaccine, large amounts of IFN-γ were generated in the supernatants of PBMC exposed for 48 h to either of the CFAs (Fig. 3). In 7 of 10 vaccinees, in vitro stimulation of PBMC by either of the CFAs resulted in considerable production of IFN-γ, particularly in response to CFA/II (arithmetic means of IFN-γ, 144 and 439 mU/mL for CFA/I and CFA/II, respectively). A vaccinee was considered to be a responder when the amounts of IFN-γ in supernatants exceeded the arithmetic mean in nonimmunized controls by at least 2 standard deviations, i.e., more than 120 and 70 mU of IFN-γ per ml in response to in vitro stimulation by CFA/I and CFA/II, respectively. Accordingly, 7 of 10 vaccinees responded with substantial IFN-γ production after only a single oral dose of the ETEC vaccine (Fig. 3).

These results were confirmed by the determination of the number of IFN-γ-secreting cells in the peripheral blood of four volunteers before and 8 days after one oral dose of the ETEC vaccine. Prior to vaccination, none of the four volunteers had more than eight IFN-γ-secreting cells per 10⁶ PBMC cocultured with either CFA/I or CFA/II. After immunization, the numbers of IFN-γ-secreting cells in PBMC cultures exposed to CFA/I and CFA/II were increased in three of four volunteers examined (Table 1). We did not find any relation between the magnitude of proliferative responses and either levels of cell-free IFN-γ (r = -0.2 [n = 10] for CFA/I) or numbers of IFN-producing cells (r = -0.03 [n = 4] for CFA/I). However, we did find a close correlation between levels of cell-free IFN-γ and numbers of IFN-γ-producing cells (r = 0.98 [n = 4] for CFA/I).

For control purposes, we also evaluated whether lipopolysaccharide (LPS) derived from the vaccine strains (i.e., purified O78 and O6 LPS [1]) could induce IFN-γ production as well as cell proliferation of PBMC isolated from nonimmunized volunteers. We were unable to evoke either type of response with LPS (data not shown). It can therefore be excluded that endotoxin contamination of the CFAs accounted for the T-cell responses recorded in this study.

**DISCUSSION**

In previous studies, we have demonstrated the appearance of vaccine-specific B cells in the blood of human volunteers orally immunized with a prototype ETEC vaccine (34). We have now examined whether oral administration of this vaccine would induce the appearance of antigen-specific T cells in peripheral blood. The two parameters most frequently used for identifying antigen-specific T cells are proliferation and production of cytokines upon in vitro culture in the presence of specific antigen. However, even though they are specifically induced, the different cytokines are not antigen specific, since their role in immune responses is often to augment and/or limit the number of effector cells in a nonspecific way.

In the present study, we have shown that oral immunization of human volunteers with the whole-cell component of a prototype ETEC vaccine induced the appearance of PBMC with the ability of proliferating in vitro when stimulated by either of two of the major protective antigens of the vaccine, i.e., CFA/I and CFA/II. Cell sorting experiments indicated that CFA-specific proliferative responses were accounted for mainly by CD4+ T cells and, to a lesser degree, by CD8+ T cells. Since IgA antibody responses are highly T cell dependent (23), it is likely that the CD4+ T cells which proliferated upon coculture with the CFAs comprised CFA-specific T-helper cells, capable of providing help to cognate B cells. We cannot rule out the possibility that the CD8+ T cells identified in this study also comprised T-helper cells, especially in view of recent reports indicating that some CD8+ T-cell clones can support the proliferation of human B cells (24) and even their differentiation into antibody-secreting cells (24, 25). Nor can we rule out the possibility that the CD4+ T cells, and the CD8+ T cells for that matter, which proliferated in response to in vitro-added CFAs also comprised suppressor and/or cytotoxic cells, as has been reported by others (15, 25, 31).

The modest T-cell proliferative responses recorded probably reflect the fact that intestinal administration of an ETEC vaccine induced only relatively small numbers of antigen-specific T-cell precursors in the circulation. Consistent with this interpretation are the facts that (i) levels of bioassayable IL-2, a major Th cell growth factor (5), were undetectable in PBMC cultures exposed to CFAs, (ii) relatively low SI (between 2 and 8) were recorded for most responders after vaccination, and (iii) large variations between SI of replicate cultures, which are generally regarded as reflecting the presence of relatively small numbers of antigen-responding T-cell precursors, were observed. Since we did not attempt to detect intracellular IL-2 or corresponding mRNA transcripts, we cannot rule out that this cytokine may in fact have been produced and immediately consumed by the same and/or other proliferating cells. Furthermore, we cannot exclude the possibility that oral ETEC vaccination may have induced a phenomenon of transient "T-cell exhaustion," seen as a decrease in IL-2 secretion in vitro by in vivo-activated T cells. Such a phenomenon has recently been described for humans receiving a parenteral influenza vaccine (13).

The main finding of this study is that oral ETEC vaccination gave rise to the appearance of T cells in blood, capable of producing IFN-γ when exposed in vitro to vaccine antigens. In
keeping with the findings of Troye-Blomberg et al. (32), no relationship between the degree of antigen-specific T-cell proliferation and IFN-γ production could be seen. Furthermore, we believe that the IFN-γ responses recorded in this study can be attributed to T cells rather than NK cells for the following reasons. First, negligible levels of IFN-γ were detected after CFA stimulation of PBMC isolated from nonimmunized individuals. Second, and more importantly, IFN-γ production increased after oral ETEC immunization in the majority of volunteers, indicating that this was an adaptive immune response and therefore did not appear to be accounted for by NK cells.

The cytokine pattern observed, i.e., no IL-2 and large quantities of IFN-γ, is in keeping with findings described by other investigators. Thus, Katz et al. (17) have shown that splenic murine T-helper cell clones specific for Bacteroides gingivalis exhibited low IL-2 activity and high IFN-γ activity. Similarly, Autenrieth et al. (3) reported the isolation of splenic murine T-cell clones specific for Yersinia enterocolitica, producing small or nondetectable quantities of IL-2 and considerable quantities of IFN-γ (500 mU/ml), comparable to the amounts of IFN-γ detected in this study. Altogether these observations call to mind recent findings demonstrating that oral immunization of human volunteers with a peroral cholera vaccine induced the appearance of large numbers of IFN-γ-secreting cells in the intestine (26). Besides its central immunoregulatory properties on lymphoid cells, IFN-γ possesses properties which may be of importance in the development of a protective mucosal immune response against a diarrheal pathogen: it stimulates B cells to secrete antibodies (27), increases the expression of secretory component on enterocytes and thus the transport of potentially protective secretory IgA (28), and regulates electrolyte secretion (12) and the permeability of the intestinal epithelium (20).

Our findings suggest that determining IFN-γ responses in peripheral blood may be more adequate than measuring the proliferation of PBMC to vaccine components for monitoring vaccine-induced T-cell activation. We found that the preimmunization levels of IFN-γ in PBMC cultures exposed to the CFAs were negligible before immunization and increased dramatically in the majority (70%) of the vaccinees after only a single oral dose of vaccine. This was confirmed by the determination of numbers of IFN-γ-secreting cells among PBMC exposed in vitro to CFAs prior to and following oral ETEC immunizations. For this reason, we propose that determination of IFN-γ levels to in vitro added vaccine antigens represents a good proxy measurement of vaccine-induced T-cell responses.

To conclude, we have demonstrated the appearance of vaccine-specific T cells in peripheral blood of orally immunized volunteers with the capacity to proliferate and to produce IFN-γ upon in vitro stimulation with vaccine antigens. We have previously shown that the prototype ETEC vaccine, when delivered orally, elicits a significant intestinal antibody response and a poor serum response (1). In other words, this vaccine appears chiefly to stimulate local mucosal immunity in the gut. We therefore propose that the responses of PBMC documented in this study were the reflection of intestinal T-cell activation. By analogy with intestinal B cells, these intestinal T cells were probably induced by oral immunization to enter the peripheral blood compartment, presumably before homing back to the intestine and other mucosal surfaces.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council grants 16X-09803 and 24X-08330, by the Göteborg Medical Society grant 92/266, by the Swedish Agency for Research Cooperation with Developing Countries, and the Faculty of Medicine, University of Göteborg.

We thank Kristina Eriksson for helpful suggestions and Margareta Fredriksson for skilful technical assistance.

REFERENCES


