Evidence for an Interleukin 4–inducible Immunoglobulin E Uptake and Transport Mechanism in the Intestine

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Summary

Immunoglobulin (Ig) E is the principal Ig involved in immediate hypersensitivities and chronic allergic diseases such as asthma. Helminths are the most potent infectious agents known for their capacity to stimulate IgE production during the course of infection. In rats, the nematode *Trichinella spiralis* typically elicits a strong parasite-specific IgE response during infection, and this IgE antibody has been shown to be protective against the parasite in passive transfer experiments. The study reported here analyzed the fate of ^{125}I-labeled myeloma IgE (1R162) in normal and *T. spiralis*-infected rats after intravenous injection. *T. spiralis* infection induced a capacity for specific binding to the gut wall of ^{125}I-IgE rather than ^{125}I-IgG1, as well as the transport of IgE, but not IgG1, into the gut lumen. Peak intestinal uptake and transport of ^{125}I-IgE occurred during the first and second weeks after injection but was not elevated in the fourth week, that is, after intestinal adult worms had been expelled. Neither ^{125}I-IgE uptake in the gut wall nor transport to the lumen could be ascribed to tissue damage or vascular leakage. Luminal transport occurred in the small intestine and not the liver, which only transports low molecular weight degraded ^{125}I-IgE. Calculations based on the amount of intact IgE in the lumen suggest that, in a 24-h period, up to 20% of injected ^{125}I-IgE can be transported to the gut lumen during the peak transport period, between 6 and 14 d after infection. The intestinal IgE binding and transport response can be adoptively transferred with *T. spiralis* immune CD4$^+$ OX22$^-$ (CD45RC$^-$) lymphocytes, which are protective, but not the nonprotective sister population CD4$^+$ OX22$^+$ (CD45RC$^+$) of lymphocytes isolated simultaneously from thoracic duct lymph of infected rats. The intravenous infusion of recombinant rat interleukin 4 also elicited significant intestinal uptake of ^{125}I-IgE. We also present evidence for the presence of CD23 on rat intraepithelial lymphocytes. These data provide evidence for a novel, inducible, intestine-specific IgE uptake and transport mechanism.

Allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and food allergy afflict up to 20% of the human population in most Western countries and are believed to be increasing in prevalence (1). Overall, allergic diseases are the most common form of human immunologic disorder; asthma is the most frequent chronic disease (2), and the only chronic disease whose prevalence is increasing despite treatment (3). The etiology of allergic reactivity and its basis in IgE-mediated pharmacologic processes of a variety of cell populations such as mast cells and eosinophils is well recognized (4, 5). The critical role of IL-4 in regulating IgE production is also now established, as are the sources of IL-4 in Th2-type T cells and mast cells (6–8). However, the factors that direct initial Th2 cell differentiation leading to IgE overproduction or that concentrate allergic reactions at mucosal surfaces are still poorly understood.

In humans living in tropical countries (9–11) or in ex-perimental animals (12, 13), high specific and nonspecific (14, 15) IgE levels are a recognized consequence of infection with helminthic parasites, particularly nematodes. Most frequently, these organisms reside in the intestine, but they may inhabit many extraintestinal sites in the body (e.g., filaria). Paradoxically, individuals chronically infected with intestinal nematodes and with high total IgE levels may have lower titers of antigen-specific IgE and less dermal allergic reactivity to common allergens than helminth-free individuals in the same environment (16). The complexity of the relationship between helminthic infection, specific IgE titers, and allergic disease, combined with the difficulty in experimentally demonstrating host protection with IgE, have raised questions regarding the protective role of IgE for the host (17, 18). Nevertheless, there is direct evidence that IgE can be protective against nematode infection in that passive transfer of purified *Trichinella spiralis*-specific IgE or IgE-rich immune serum in conjunc-

tion with *T. spiralis*-specific T cells will lead to rejection of the parasite (19). Indirect evidence also exists for a role of IgE in schistosome infections based on in vitro experiments and field epidemiological data (20–22). Despite this growing body of evidence that attests to a protective role of IgE, no in vivo effector mechanism involving IgE has been defined for any of these parasites. The site of protection during IgE-mediated rapid expulsion of *T. spiralis* is believed to be the mucosal epithelial layer, the site of larval residence. During a challenge infection with *T. spiralis*, the transfer of IgE-rich immune serum can be delayed for up to 6 h after the larval parasite has entered its intestinal niche in epithelial cells and still elicit rapid expulsion of the parasite (23). This evidence suggests that IgE-mediated processes induced by the parasite take place in the outer cellular layer, comprising the epithelial cells (EP)[1] and intraepithelial lymphocytes (IEL), rather than in the gut lumen. Reactions taking place in the lamina propria (LP) or beneath the basement membrane, which could involve mast cells, are also possible candidates for effector processes. To delineate potential sites of function of IgE against *T. spiralis*, we used the passive transfer of ^{125}I-IgE to monitor the systemic and intraintestinal behavior of IgE in *T. spiralis*-infected rats. We also used infection-free systems based on the transfer of activated *T. spiralis* immune CD4$^+$ cells and recombinant rat IL-4. The data show that there is an inducible IgE-specific uptake and transport system in the gut, which results in the passage of intact, high molecular weight plasma IgE into the gut lumen.

Materials and Methods

Animals and Infection. Adult male or female AO rats 6–8 wk of age and weighing 150–250 g raised in the J.A. Baker Institute vivarium were used in these experiments. Food and water were provided ad libitum. In any given experiment, rats of only one sex were used. The parasite, *T. spiralis*, was maintained in the laboratory by serial passage in irradiated rats. For infections, 2,000 infective larvae isolated by pepsin-hydrochloric acid digestion of infected muscles were given orally to rats (24).

Radiolabeling of IgE and IgG1. Rat myeloma IgE (IR162), prepared as previously described (25), and affinity-purified normal serum IgG1 were labeled with ^{125}I (Amersham Corp., Arlington Heights, IL) by the iodogen method (26). For labeling, ~1 mCi of ^{125}I was used per milligram of protein. The reaction took place in 100–200 µl of Dulbecco's PBS (DPBS) using from 100 to 200 µg IgE or IgG1. After iodination, samples were loaded on to a desalting column (GF-5; Pierce Chemical Co., Rockford, IL) to collect the high molecular weight fraction according to the manufacturer's instructions. Free label comprised <5% of the final labeled protein. The purity and molecular integrity of the labeled protein was checked by running the samples in a nondenaturing gel followed by autoradiography. Specific activity (cpm/milligram protein) was calculated from the total ^{125}I count of the samples and their known protein concentration. For tracer studies, 10–20 µg of radiolabeled protein (~500,000–700,000 cpm) was injected intravenously through the tail vein of each rat.

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[1] *Abbreviations used in this paper:* CM, conditioned media; DPBS, Dulbecco's PBS, EP, epithelial cells; FBS, fetal bovine serum; IEL, intraepithelial lymphocytes; LP, laminia propria; RBL, rat basophilic leukemia cells; SAS, saturated ammonium sulphate.

Affinity Chromatography. Affinity columns were used to purify *T. spiralis* antigen (TSL-1) defined by monoclonal antibody 9D4 (27), rat anti–*T. spiralis* IgE, and IgG1 from normal rat serum as previously described (28). The monoclonal antibodies used [anti TSL-1, 9D4; A2 anti–rat IgE (29) and RG11/39 anti–rat IgG1 (30) were precipitated from ascitic fluid by 40% saturated ammonium sulphate (SAS) treatment. The dialyzed antibodies (0.1 M sodium bicarbonate and 0.5 M sodium chloride, pH 8.0) were coupled to cyanogen-bromide–activated Sepharose 4B gel (Pharmacia Fine Chemicals, Piscataway, NJ), washed, and poured into a column. After prewashing the column, the antigen or antibody mixture was applied in borate-buffered saline (0.1 M boric acid plus 0.3 M sodium borate in 0.85% saline, pH 8.3) and eluted with glycine/HCl buffer (0.05 M glycine in 1 M NaCl, pH 2.5). The acid eluate was concentrated by negative pressure while being dialyzed against DPBS, then stored frozen at −70°C until use.

Collection of Tissue and Body Fluids. In experiments examining ^{125}I-IgE or ^{125}I-IgG1 half-life in serum, blood samples (0.5 ml) were collected from each rat at 5 min and thereafter at intervals of 6 h after injection of ^{125}I-IgE or ^{125}I-IgG1. In most other experiments, animals were euthanized 24 h after ^{125}I-IgE injection, and tissues and body fluids were taken for weighing and counting. In some experiments, this involved collecting all tissues and organs of the body except the head. Small-intestinal fluid was obtained by flushing the lumen with 30 ml PBS, but the large intestine was counted with its contents intact. In experiments that monitored total body loss of radioactive counts, animals were housed individually to collect urine and fecal samples during the 24-h period. In these experiments, counts of samples of muscle, skin, and bone were multiplied by the percent contribution to total body weight of that tissue based on the data of Foster and Frydman (31). Radioactive counts in individual tissues or fluids were measured by use of a gamma counter (γ-8000; Beckman Instruments, Inc., Fullerton, CA). Depending on the experiment, ^{125}I-IgE localization is expressed as total organ count or cpm/gram of tissue.

Bile Duct Cannulation. Bile duct cannulation was performed aseptically as described by Waynforth and Flecknell (32). Briefly, after opening the abdominal wall, a short section of bile duct above the entry of the pancreatic ducts was cleared of most of the surrounding tissue and loosely ligatured anteriorly and distally. A transverse cut was made between the ligatures, and a 0.5-mm polyethylene cannula was introduced into the bile duct and tied in place. The distal end of the cannula was brought out of the lateral peritoneal wall. After surgery, the animals were housed in individual restraining cages, and bile was collected every 2.5 h on a fraction collector (Trac-300; Pharmacia Fine Chemicals). In some experiments, bile was collected in tubes that contained 3 ml SAS to precipitate proteins in bile as they were collected.

Adoptive Transfer of Cells. Thoracic duct lymphocytes were obtained from rats 3 d after infection by cannulation of the thoracic duct and collection of lymph for 20–24 h (33). Cells were washed twice in RPMI 1640 medium (GIBCO BRL, Gaithersburg, MD) with 5% fetal bovine serum (FBS; Harlan Bioproducts for Science, Inc., Indianapolis, IN) and separated into different subsets (B cells, CD8$^+$, CD4$^+$, CD45RC$^+$, and CD4$^+$ CD45RC$^-$) by panning as described earlier (34). Antibodies used for panning were affinity-purified sheep anti–rat F(ab')$_2$ made at the Baker Institute; mouse anti–rat CD8 (OX8) mAb (35); mouse anti–rat CD45RC (OX22) mAb (36), and affinity-purified sheep anti–mouse Ig (25 µg/ml) produced at the Baker Institute. In transfer experiments, 2 × 10^8 CD4$^+$ CD45RC$^+$ or CD4$^+$ CD45RC$^-$ cells were injected intravenously through the tail vein.

Detection of IgE in Intestinal Wash Fluids and Bile. Radioimmu-

noassay was performed in Costar 96-well plates (Fisher Scientific Co., Pittsburgh, PA) using a modified ELISA technique. Briefly, the wells were coated overnight at 4°C with the anti–rat IgE antibody A2, and the vacant binding sites were blocked with 1% BSA (Sigma Chemical Co., St. Louis, MO). 100 μl intestinal wash fluid or bile sample was transferred to triplicate wells and incubated at room temperature for 1 h. After incubation, the wells were washed three times with PBS and counted in a gamma counter. Serial dilutions of ^{125}I-IgE of known concentration were used as standards. To determine whether intestinal wash fluids or bile contained enzymes that degraded IgE, a known amount of ^{125}I-IgE was added to some intestinal fluid or bile samples and incubated for 1 h at room temperature. Recoverability of the added ^{125}I-IgE was then measured by radioimmunoassay.

Electrophoretic Separation of Proteins in Intestinal Wash Fluids. To determine the presence of IgE in the intestinal lumen, proteins in intestinal wash fluids were precipitated first with 40% SAS to remove IgG and other proteins and centrifuged at 1,000 rpm in a 7″ rotor for 10 min in a centrifuge (Sorvall RT 6000; DuPont, Boston, MA). The supernatant was reprecipitated with SAS at a final concentration of 60% for 1 h at 4°C and centrifuged at 12,000 rpm in a 2″ rotor for 10 min in a microfuge (Beckman Instruments, Inc.). The supernatant was removed and the precipitate resuspended in 20 μl PBS and counted in a gamma counter. For electrophoretic separation, the precipitates were then diluted with sample buffer, and equal counts were loaded onto an 8–20% gradient gel (Phast system; Pharmacia Fine Chemicals). The proteins were separated under nondenaturing conditions. After the run, the gels were stained, dried, and autoradiographed.

Collection of EP, IEL, and LP Cells. EP, IEL, and LP cells were isolated from the intestine by standard procedures (37, 38). After removing the Peyer's patches and mesentery, the intestine was split open longitudinally, cut into small pieces (2–5 cm) and incubated for 30 min at 37°C in 1.3 × 10^{-4} EDTA (in Ca^{2+} Mg^{2+} free HBSS), and then vortexed briefly. This procedure separated the EP and IEL. The cell suspension was then collected and passed through a glass wool column to remove mucus and debris. The eluate was then layered over 35% Percoll in HBSS and spun at 400 g for 20 min. EP were collected from the interphase and IEL from the bottom of the tubes.

To recover LP cells, intestinal tissue remaining after removal of the epithelial layer was incubated for 4 h at 37°C in RPMI 1640 containing 25 mM Hepes (Sigma Chemical Co.), 0.01% collagenase (GIBCO BRL), 0.01% deoxyribonuclease (Sigma Chemical Co.) and 0.01% soybean trypsin inhibitor (Sigma Chemical Co.). After incubation, the suspension was passed through gauze to remove mucus and debris. The cells in the suspension were sedimented by centrifugation at 200 g for 10 min, resuspended in 30% Percoll in RPMI, and layered over 67.5% Percoll. The gradient was then centrifuged at 600 g for 20 min and the cells at the interface collected. This procedure separated mast cells, which pass through the 67.5% Percoll layer (38), from the rest of the LP cells. After separation, the EP, IEL and LP were washed twice in RPMI containing 10% FBS, their viability checked by trypan blue dye exclusion, and their purity determined after staining cytospin preparations. The IEL preparation had 1–5% EP contamination, and the LP preparation contained no toluidine blue–staining cells.

Binding Assays. To measure ^{125}I-IgE binding to cells, 10^6 EP, IEL or LP cells suspended in 200 μl RPMI were incubated with 2 μg ^{125}I-IgE for 2 h at 37°C. After incubation, 100 μl of the cell suspension was layered over 200 μl of phthalate oil (60% dibutyl phthalate, 40% Bis 2-ethylhexyl phthalate; Eastman Kodak Co., Rochester, NY) and centrifuged at 10,000 rpm for 1 min. The

aqueous phase with the free label remained on top of the phthalate oil and the cells passed through. The fluid phase was then aspirated off, and the tip of each tube was snipped off to count the cell-associated radioactivity in a gamma counter. The specificity of IgE binding was determined by incubating target cells with cold IgE (20–50 μg/10^6 cells) for 1 h before adding ^{125}I-IgE (2 μg). To identify the type of receptor involved in the binding of IgE, cell aliquots were incubated for 2 h with anti–rat FcεRI antibody [Cd3; a gift from Dr. D. Holowka, Cornell University, Ithaca, NY (39)] at a concentration of 3 μg/10^6 cells in 100 μl or with polyclonal rabbit anti-murine FcεRII antibody (anti-murine CD23 but cross-reactive with rat; a gift from Dr. D. Conrad, Virginia Commonwealth University, Richmond, VA) at a concentration of 20 μg or 50 μg/10^6 cells in 100 μl before addition of ^{125}I-IgE. Rat basophilic leukemia (RBL-H2) cells and rIL-4–stimulated (for 24 h) spleen B cells were used as positive controls for FcεRI and FcεRII, respectively.

Conditioned Media. Thoracic duct lymphocytes from rats infected 3 d previously with *T. spiralis* were separated into CD4$^+$ CD45RC$^+$ and CD4$^+$ CD45RG$^-$ cells by panning (33). After separation, the cells were resuspended at 2 × 10^7 cells/ml in RPMI medium containing 2 mM glutamine (GIBCO), 0.04% NaHCO$_3$ (Sigma Chemical Co.), 1 mM sodium pyruvate (GIBCO), 25 mM Hepes, 50 mM 2-ME (Fisher Scientific Co.), 15% heat-inactivated FBS, 10 μg/ml gentamicin, and 100 μg/ml *T. spiralis* antigen that had been affinity purified on a 9D4 column (28). The cell suspension was then transferred (1 ml/well) into 6-well culture plates (Corning; Fisher Scientific Co.) and incubated for 24 h at 37°C with 5% CO$_2$ in air. Culture supernatants [conditioned media (CM)] collected after 24 h incubation were filtered through a 0.22-μm filter (Costar Corp., Cambridge, MA), aliquoted, and stored at -70°C until use.

In Vivo Recombinant IL-4 Treatment. In these experiments, three rats were injected intraperitoneally with 2.5 ml culture supernatant of rat IL-4–transfected CHO cells (40). This supernatant was generously donated by Dr. A. McKnight (Oxford University, Oxford, U.K.). IL-4 activity in the CHO supernatants was confirmed by upregulation of class II expression on splenic B cells, whereas the mock CHO supernatants had no effect (40). CHO-IL-4 supernatant consistently induced IgE class switching in LPS-stimulated rat splenic B cells, whereas the mock CHO supernatant did not (41). Three control rats received 2.5 ml culture supernatant from mock-transfected CHO cells. 3 d later, 6.49 μg of ^{125}I-IgE (sp act 77,000 cpm/μg protein) was injected intravenously through the tail vein, and the tissues and body fluids were collected as above. In addition, we tested the capacity of antigen-stimulated CM from CD4$^+$ OX22$^+$ and CD4$^+$ OX22$^-$ cells. Three rats were injected intraperitoneally with 5 ml each of the respective CM and injected 3 d later with ^{125}I-IgE in parallel with the rats receiving r.IL-4.

Statistical Analysis. All values are expressed as the mean ± SD. The statistical significance of the means of comparable groups was tested by use of a one-way analysis of variance. Probability values $\leqslant$5% were considered significant.

Results

Tissue Distribution of ^{125}I-IgE in T. spiralis–infected Rats. Infection with *T. spiralis* induces a strong T cell response that results in elevated IgE levels (19). The IgE-dependent immune process leading to elimination of larval *T. spiralis*, rapid expulsion, is expressed by the end of the 1st wk after infection and reaches its peak around 4 wk after infection. To analyze the behavior of IgE during this period, ^{125}I-IgE was in-

Table 1. *Recovery of ^{125}I Counts from Different Organs/Fluids of Rats 24 h after Intravenous Infusion of 20.65 µg ^{125}I-IgE (sp act 24,216 cpm/µg IgE) through the Tail Vein*

Tissue	Infected	Control
Lung	5,278 ± 237*	1,648 ± 209
Small intestine	12,034 ± 4,739‡	3,712 ± 1,130
Large intestine	4,957 ± 742‡	2,907 ± 169
Mesenteric lymph nodes	2,557 ± 836‡	1,216 ± 114
Intestinal wash fluids	27,882 ± 7,264‡	1,842 ± 856
Other intestine (cecum and stomach)	46,086 ± 8,506	45,812 ± 9,481
Other viscera (liver, spleen, kidneys, heart, and peritoneum	21,685 ± 1,734	20,998 ± 1,238
Thyroid	5,131 ± 347	4,994 ± 215
Muscle (40% of total mass)§	27,994 ± 7,340	32,537 ± 4,955
Skin (12% of total mass)§	38,474 ± 4,978	41,574 ± 5,937
Urine and feces	204,291 ± 49,198	227,252 ± 53,277
Total recovered	396,327 ± 57,368	384,503 ± 66,004

Rats were infected 7 d earlier with 2,000 muscle-stage larvae of *T. spiralis.*
Significant at $p <$* 0.05; ‡ 0.01.
§ Estimate based on Foster and Frydman (31); n = 4.

jected at different time points after infection. A typical experiment (Table 1) shows a significant ($p \leq 0.01$) increase in the recovery of ^{125}I from the lung, small intestine, large intestine, mesenteric lymph nodes, and small-intestinal wash fluid of rats infected 7 d previously with *T. spiralis*, compared with uninfected control animals. In this experiment, the total body distribution of ^{125}I-IgE was determined quantitatively by sampling every organ and collecting feces and urine. The results are expressed as total counts per organ to reflect the overall distribution of ^{125}I-IgE. Approximately 80% of injected counts were recovered from both experimental and control groups. However, the proportion that entered the gut (total gut = stomach, small and large intestine, cecum, intestinal wash, and feces) was 50% higher in infected rats than in controls, with the small intestine ^{125}I count accounting for 86% of this difference. The primary site of disposal of ^{125}I-IgE was the urine, which accounted for 36–41% of total counts after 24 h.

To analyze whether the distribution of IgE in the intestine was altered throughout the course of infection, we measured 24-h ^{125}I-IgE tissue recovery from rats that had been infected 1, 2, 3, or 4 wk previously. Increased recovery of radiolabel in the intestinal tissue and fluids was significant during the first 2 wk after infection but usually not at ≥ 3 wk (Fig. 1). These results were reproducible in infected animals 4–14 d after infection, but were more variable after 2 wk in that intestinal ^{125}I-IgE uptake was sometimes still elevated at 3 wk. Further analysis showed that ^{125}I-IgE counts peaked in the small intestine 4–6 d after infection (data not shown).

Specificity of Intestinal Uptake of IgE. Previous work had shown that IgE and IgG1 would both transfer rapid expulsion of *T. spiralis* to the intestine under appropriate, but different, conditions (19, 42). We therefore determined whether

IgG1 behaved like IgE in intestinal tissue. The presence of equivalent amounts of ^{125}I-IgG1 or ^{125}I-IgE might indicate that parasite-induced damage or inflammation caused a generalized leakage of plasma proteins into the gut. To do this, we injected ^{125}I-IgE or -IgG1 in comparable amounts into different rats infected 2 or 4 wk earlier. Significantly elevated
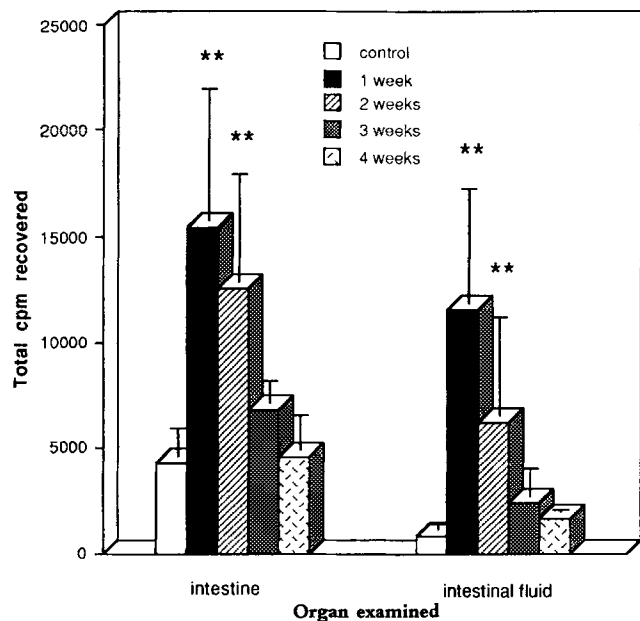

Figure 1. Uptake of ^{125}I-IgE in the small-intestinal wall and its transport to the intestinal lumen of rats infected with 2,000 muscle-stage larvae of *T. spiralis* 1, 2, 3, or 4 wk earlier. The counts were made 24 h after intravenous transfer of ~12 µg ^{125}I-IgE (sp act 42,000 cpm/µg protein). **Significant at $p < 0.01$ compared with uninfected controls. Groups of four rats ± 1 SD.
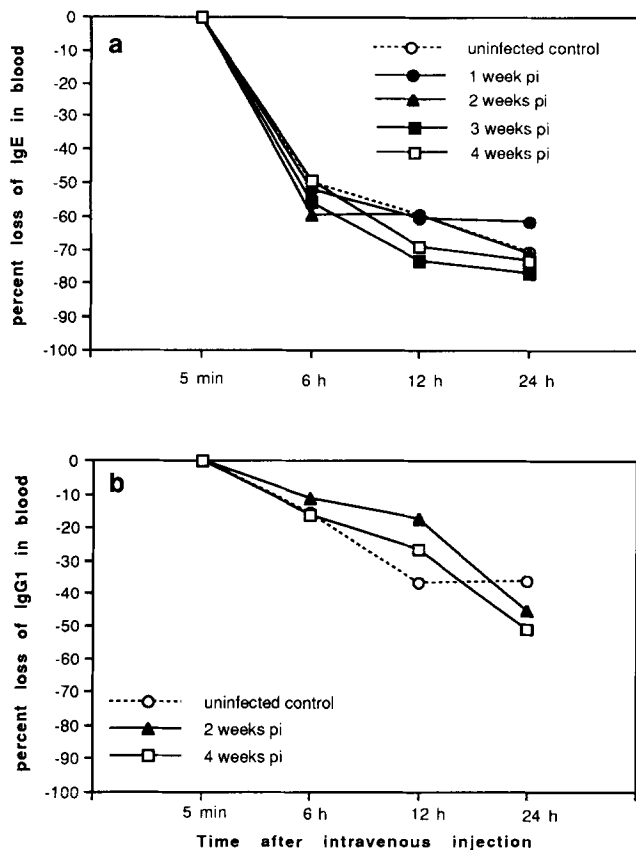
Figure 2. Rate of loss of (a) ^{125}I-IgE or (b) ^{125}I-IgG1 from the blood in rats over 24 h after intravenous injection. Points represent means of three rats that were infected 1, 2, 3, or 4 wk before and controls. None were significantly different from controls.

^{125}I-IgE levels were found 24 h later in the small intestine (12,517 ± 5,086), mesenteric lymph node (1,136 ± 608), and lungs (2,389 ± 562) compared with uninfected controls in the 2 wk–infected groups (1,355 ± 1,275; 282 ± 87; and 358 ± 209, respectively). However, there was no significant increase in ^{125}I-IgG1 counts in any organ of infected rats at either 2 or 4 wk after infection. This was true whether the counts were expressed as total counts per organ or cpm per gram of tissue (data not shown). Intestinal wash counts at 24 h in ^{125}I-IgG1 recipients were 1,113 ± 664 (2 wk) versus control, 1,216 ± 472 (not significant) whereas for IgE recipients, the counts were 6,239 ± 4,556 (2 wk) and control, 826 ± 254 (p <0.05).

The Half-Life of ^{125}I-IgE in Blood. *T. spiralis* infection induces elevated serum IgE levels (19) and, as the above data suggest, increased levels of IgE in the gut. Under these conditions, it seemed possible that the rate of IgE clearance from serum could be reduced as has been found in patients with hyperimmunoglobulin E syndrome (43). In normal rats, the half-life of IgE in serum is ~12 h (44) but it has never been estimated in experimental animals infected with nematodes.

In *T. spiralis*–infected rats, the half-life of ^{125}I-IgE in circulating blood was calculated by use of the 5-min blood value as the point of equilibration of extravascular and intravascular pools (0% loss). Approximately 50–60% of the total
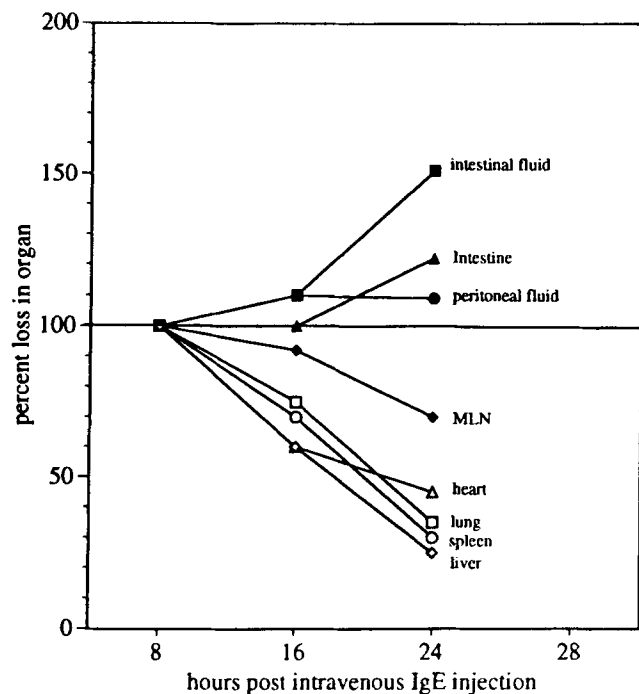

Figure 3. Kinetics of accumulation of ^{125}I counts in various organs of rats injected with ^{125}I-IgE 7 d after infection with *T. spiralis*. Data are the means of four rats per time point. The 8-h time point has been normalized to 100% for each organ, and subsequent counts are expressed as the percentage of the 8-h count.

label was lost from the blood 6 h after intravenous injection in both infected and uninfected animals (Fig. 2 a). By 24 h, the loss was 70–77%. There was no difference in the rate of loss of ^{125}I-IgE between infected or uninfected animals. In contrast, the loss of ^{125}I-IgG1 from the blood, also based on the 5-min value as the point of equilibration, showed that only 10–15% of counts were lost by 6 h and 38–50% by 24 h in both infected and uninfected animals (Fig. 2 b).

Kinetics of IgE Uptake in Various Organs of T. spiralis–infected Rats. The above experiments detailing body distribution of ^{125}I-IgE had all been conducted 24 h after the injection of labeled Ig. To analyze the behavior of transferred IgE in infected rats during the first 24 h after injection, groups of four rats were killed at 8, 16, and 24 h after ^{125}I-IgE transfer. ^{125}I-IgE counts in each organ during the 24-h period after injection of ^{125}I-IgE showed a characteristic loss of label with time from most organs (heart, lung, liver, spleen). In contrast, ^{125}I-IgE accumulated during the 24-h period in the intestine, intestinal fluid, and peritoneal fluid (Fig. 3). These observations are significant, as all other tissues displayed a 25–75% decrease in label recovery by 24 h after injection compared with the normalized 8-h value. The rate of decline in counts associated with the heart, liver, lung, and spleen paralleled that previously found in the blood.

Molecular Weight of Luminal ^{125}I-IgE. High ^{125}I counts in the intestinal lumen suggested that IgE might be transported to this site or that it could be a site of degradation of plasma IgE. To analyze this, we determined the molecular weight of labeled material in the lumen of infected rats whose bile

ducts had been cannulated and exteriorized. Bile duct cannu-
lation was used to derive an independent estimate of possible
transport of IgE via the liver. The rats were injected with
10 μg ^{125}I-IgE (sp act 77,000 cpm/μg) intravenously im-
mediately after cannulation, and the bile was collected for
a 25-h period at 2.5-h intervals. A mock bile duct cannula-
tion through a laparotomy was performed on control infected
and uninfected rats. When killed at 25 h, a significant (p
<0.01) and equal increase in ^{125}I counts in the small intes-
tine and small-intestinal wash was evident in both bile
duct–cannulated and bile duct–intact infected rats, compared
with uninfected controls. To ascertain the proportion of ^{125}I
counts in the intestinal wash fluid or bile that were protein
bound, we precipitated these samples sequentially with 40
and 60% SAS. The intestinal wash (60% SAS precipitate)
of bile duct–cannulated infected animals contained 4,004 cpm
that were protein bound (0.5 ± 0.1% of that infused), whereas
the bile duct of cannulated uninfected animals had 146 cpm
in the precipitated material (not above background 152 cpm).
In bile duct–intact infected animals, the recovery of protein-
bound counts in the intestinal fluids was 5,244 cpm (0.7 ±
0.1%) of that infused. In bile duct–intact uninfected animals,
the recovery of protein-bound intestinal wash counts was at
background levels. Overall, from 9 to 29% of the intestinal
wash counts recovered from infected animals were due to
immune-reactive precipitable IgE. No counts were precipitable
in the bile of either infected or uninfected animals. When
the precipitated proteins from intestinal washes were run on
a nondenaturing gel, high molecular weight intact IgE was
detected in the intestinal wash fluids (Fig. 4) of bile duct–in-
tact or bile duct–cannulated rats. Only low molecular weight
^{125}I material migrating with the dye front was detected in
bile (unprecipitated sample).

Radioimmunoassay for IgE in Intestinal Wash Fluids and Bile.
To confirm the presence of IgE in intestinal wash fluids,
an ELISA-based radioimmunoassay was performed. Using
^{125}I-IgE of known specific activity as the standard, we used
a capture ELISA to measure the amount of IgE in intes-
tinal wash fluids and bile. This assay will measure down to
~5 ng/ml IgE. The results showed that 79.5 ± 45.1 ng IgE
(0.8% of that injected) and 61.1 ± 23.3 ng IgE (0.6% of
that injected) was present in the intestinal wash fluids of bile
duct–cannulated and bile duct–intact *T. spiralis*–infected ani-
mals, respectively, 24 h after intravenous injection of ^{125}I-IgE.
Uninfected rats had <5 μg/ml IgE in intestinal washes. Al-
though significant ^{125}I counts were present in bile of both
infected and uninfected animals (data not shown), no IgE
could be detected in bile by radioimmunoassay (data not
shown). Over 94% of the spiked ^{125}I-IgE added to normal
samples was measured by ELISA in both intestinal fluids
(94 ± 1.5%) and bile (97 ± 1.1%), suggesting minimal degra-
dation of the protein after 1 h incubation at room temperature.

Intestinal Cell Binding of IgE. The transport of IgE to the
intestinal lumen, demonstrated above, suggested cellular
binding of free ^{125}I-IgE in the intestine. To determine which
cell populations were involved, we first examined sectioned
material by autoradiography. This was unsuccessful because
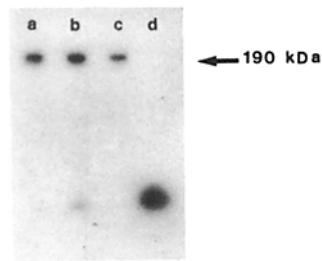of the low counts of thin paraffin sections. An alternative

Figure 4. Detection of intact
IgE in the intestinal lumen. Elec-
trophoretic separation of proteins
in small intestinal wash fluids and
bile of rats that received ^{125}I-IgE
(sp act 77,000 cpm/μg) intrave-
nously 24 h earlier. Equal ^{125}I
counts of proteins that had been
precipitated with 60% SAS were
separated in an 8–20% gradient
gel under nondenaturating con-
ditions. After the run, the gel was
dried and autoradiographed. (Lane *a*) ^{125}I-IgE that was used for infusion;
(lane *b*) 60% SAS precipitate of the small-intestinal fluids of sham-operated
rats; (lane *c*) 60% SAS precipitate of the small-intestinal fluids of bile-
duct–cannulated rats; (lane *d*) nonprecipitated bile. Molecular weights of the
proteins were calculated from standards run parallel to the samples. The
low molecular weight labeled material in lanes *b* and *d* is migrating with
the dye front.

approach was to isolate various intestinal cell populations from
infected rats and measure their capacity for ^{125}I-IgE uptake
in vitro. Isolated cell populations from the gut of infected
and uninfected rats were incubated with ^{125}I-IgE. Increased
binding of IgE to IEL and LP cells collected from *T. spiralis*–
infected animals compared with uninfected controls was evi-
dent (Table 2). However, in two separate experiments, it was
difficult to reproducibly measure IgE binding to enterocytes
from infected animals because of heavy mucus production
induced by the infection. In all cell populations from infected
rats, unlabeled IgE inhibited binding by ≥80%; only unin-
fected IEL did not show inhibition.

To further characterize the receptor(s) involved in the
binding of ^{125}I-IgE to IEL or LP, the cells were incubated
with antibodies against FceRII (mAb Cd3) or FceRI (poly-
clonal anti-CD3) before the binding assay in the above ex-
periment. Prior incubation of cells with Cd3 reduced ^{125}I-IgE
binding to LP cells by 40% and also blocked binding of
^{125}I-IgE to RBL (Table 2) by 81%. Prior incubation of cells
with anti-CD23 at 20 μg/10^6 cells significantly blocked
^{125}I-IgE binding to LP (42 ± 18%; p ≤0.05) and IEL (87 ±
4%; p ≤0.01). At a concentration of 50 μg/10^6 cells, anti-
CD23 blocked ^{125}I-IgE binding to LP by 95.3% (p ≤ 0.01)
in cells collected from *T. spiralis*–infected animals (Table 2).
No significant blocking was observed with IEL from con-
trol animals, but high level blocking occurred with LP cells.
Anti-CD3 also significantly (35 ± 0.1%; p <0.05) blocked
binding of ^{125}I-IgE to IL-4–stimulated rat spleen B cells (data
not shown).

*IgE Uptake in the Intestine is Upregulated by Adoptive Transfer
of Immune T Cells.* The above data indicated that an intesti-
nally based IgE uptake and transport process could be in-
duced by a *T. spiralis* infection. Previously, we demonstrated
that passively transferred immune IgE was unable to confer
protection unless a population of activated thoracic duct
CD4$^+$ OX22$^-$ (CD45RC$^-$) T cells was transferred before
the IgE (19, 45). To determine whether intestinal IgE uptake
might be induced by the reactive CD4$^+$ cells that transfer
IgE-dependent worm expulsion, we measured IgE uptake in
the gut of naive rats 7 d after adoptive transfer of 3-d CD4$^+$
CD46RC$^-$ (protective) or CD4$^+$ CD45RC$^+$ (nonprotec-

Table 2. *Role of Specific IgE Receptors in* ^{125}I-IgE *Uptake by Intestinal Cell Populations*

		^{125}I-IgE	^{125}I-IgE + cold IgE (50 μg)	^{125}I-IgE + anti FcεRI (20 μg)	^{125}I-IgE + anti FcεRII (50 μg)
Enterocytes	Infected	2,776 ± 871	ND	ND	1,658 ± 138
	Control	1,528 ± 421	761 ± 253	1,350 ± 465	1,471 ± 272
IEL	Infected	2,949 ± 509*	265 ± 109	2,225 ± 614	373 ± 230‡
	Control	1,567 ± 350	1,494 ± 470	1,452 ± 256	1,053 ± 38
LP	Infected	2,369 ± 30*	339 ± 89‡	1,386 ± 180‡	141 ± 29‡
	Control	1,657 ± 138	414 ± 69‡	1,309 ± 150	158 ± 12‡
RBL		3,038 ± 50	226 ± 62‡	574 ± 39‡	3,116 ± 35

Cell populations were isolated from rats infected 10 d earlier and immediately used in assays. RBL cells were grown from stock for 72 h in vitro before use. Significance comparisons are for * infected vs. control animals or ‡ test inhibition (IgE or antibody) vs. ^{125}I-IgE (column 1). All probabilities are ≤0.05. Values without an asterisk are not significant. Assays done in triplicate.

tive) cells. When measured 24 h after the intravenous injection of ^{125}I-IgE, there was a significant (p ≤0.01) increase in the recovery of radiolabel from the intestine and mesenteric lymph nodes of rats that received CD4$^+$ CD45RC$^-$ cells compared with those that received CD4$^+$ CD45RC$^+$ cells or no cells (Fig. 5). A consistent finding was a small but significant increase in recovery of radiolabel in the intestinal fluid of animals that received CD4$^+$ CD45RC$^-$ cells.

IgE Uptake Can Be Induced by r.IL-4 Treatment. Earlier experiments demonstrated that CD4$^+$ CD45RC$^-$ cells isolated from the thoracic duct of rats 3 d after infection secreted substantial amounts of IL-4 (41). To determine whether changes in IgE distribution could be produced by IL-4, we infused rats with rat r.IL-4 (40) and conditioned medium from in vitro–stimulated CD4$^+$ OX22$^+$ and CD4$^+$ OX22$^-$

cells and then injected ^{125}I-IgE 3 d later. The results (Fig. 6) showed a significant increase in total small-intestinal ^{125}I counts of 10,945 ± 918 (IL-4 recipients) versus 6,065 ± 1,338 (mock supernatant) and intestinal wash counts 3,416 ± 852 (IL-4) vs 1,042 ± 27 (mock supernatant). There was no overall increase in intestinal ^{125}I-IgE uptake in recipients of supernatants from CD4$^+$ CD45RC$^-$ or RC$^+$ cells stimulated in vitro, but there was an 80% increase (1,402 ± 213 cpm vs. 777 ± 158 cpm) in total ^{125}I counts in the small-intestinal wash of rats that received CM from CD4$^+$ CD45RC$^-$ cells compared with CM from CD4$^+$ CD45RC$^+$ cells. No significant differences were observed in the recovery of ^{125}I counts in other organs or body fluids between the two groups.
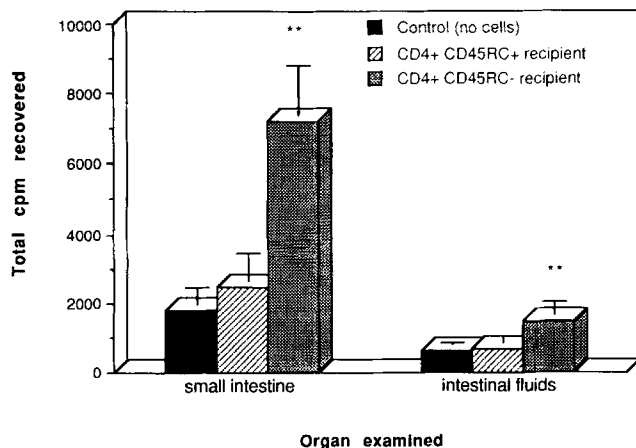

Figure 5. Uptake of ^{125}I-IgE in the small-intestinal wall and its transport into the intestinal lumen of rats that received 2 × 10^8 protective (CD4$^+$ CD45RC$^-$) or nonprotective (CD4$^+$ CD45RC$^+$) cells. 7 d after adoptive cell transfer, 12 μg ^{125}I-IgE (sp act 42,000 cpm/μg protein) was injected intravenously through the tail vein, and 24 h later, ^{125}I counts in the tissue and body fluids were measured. **Significant at p <0.01 compared with sham-operated controls. Groups of four rats ± 1 SD.
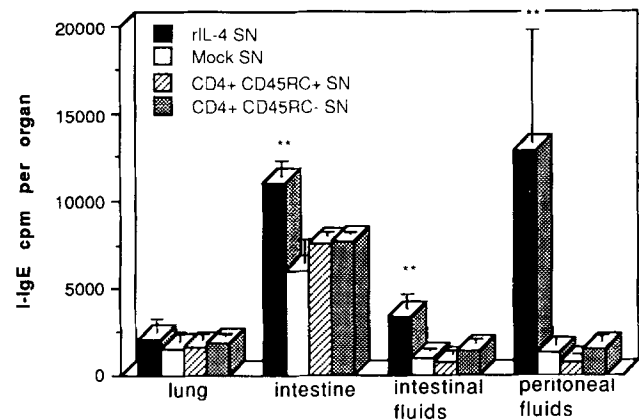

Figure 6. Uptake of ^{125}I-IgE (sp act 77,000 cpm/μg) in the small intestine of rats treated 3 d previously with an intraperitoneal injection of a culture supernatant from rat r.IL-4–transfected CHO cells, mock-transfected CHO cells, or TSL-1 antigen–stimulated CD4$^+$ (CD45RC$^+$ or CD45RC$^-$) cells. The CD4$^+$ subsets were collected from the thoracic duct of rats infected 3 d earlier with *T. spiralis*. After collection, the cells were panned and stimulated in vitro with TSL-1 (100 μg/ml) for 24 h. IL-4 activity in transfected CHO cell supernatants and in CD4$^+$ subset supernatants was determined earlier by appropriate bioassays. Significant at *p <0.05% or **p <0.01% compared with mock-transfected supernatants. Groups of four rats ± 1 SD.

Discussion

Despite its prominent pathological effects, it has proved difficult to establish a beneficial role for IgE. Over the last few years, both direct and indirect evidence has accumulated suggesting that protection against various helminth infections can be attributed to IgE (19–22), through as yet undefined mechanisms. Unique biological features of IgE include its low concentration in serum, a conspicuously short half-life in plasma (12 h), but a long half life (7.4 d) (44) when bound to the high affinity receptor (FcεRI) now known to be expressed on a variety of cells (20, 46, 47). Cell binding appears to be a prerequisite for function, and the short plasma half-life is thought to be due to the presence of two catabolic pathways that act on free IgE (48). The results of this study further our understanding of IgE by demonstrating an inducible capacity for uptake of plasma IgE by intestinal cell populations, that intact IgE is preferentially transported into the intestinal lumen, and that gut uptake of IgE can be induced by the adoptive transfer of purified T. spiralis immune CD4$^+$ OX22$^-$ cells or the intravenous injection of rat r.IL-4.

Previous studies have suggested that IgE may be a secretory immunoglobulin (49) based on the simple detection of IgE in intestinal fluids (49–52) or respiratory and nasal washings (49, 53–55). The ratio of IgE to total protein or serum albumin indicated that more IgE was present in gut an lung fluid than could be accounted for by diffusion from serum (49, 54, 55). However, questions regarding the radioimmunosorbant test IgE assay system used (56) and the inducible secretion of IgG in pancreatic fluid (51) cast doubt on the view that IgE was a secretory immunoglobulin. Furthermore, no evidence for IgE secretion was found in a quantitative study of the perfused gut of normal human volunteers in which IgG, IgA (polymeric and monomeric), IgM, albumin, and orosomucoid levels were measured (57). IgE in secretions is not combined with secretory component (58) nor does IgE combine with secretory component in vitro (59). Thus, if IgE is a secretory Ig, then its mechanism of uptake and transport will likely differ from the poly-Ig receptor mechanism of sIgA (60). Most observers have associated elevated IgE levels in mucosal fluids with underlying inflammatory or allergic processes and have suggested that diffusion from an enriched local source of IgE-secreting plasma cells could account for their findings (49, 51, 54, 55). This is consistent with a concentration of IgE-containing cells in the intestinal and respiratory mucosae and their draining lymph nodes in primates and parasite-infected rodents (61, 62). However, this conclusion has also been challenged, by studies that have failed to find IgE-containing cells in the intestinal LP (63) and evidence that many IgE-bearing cells in the gut of nematode-infected rats were mast cells (64, 65). Thus, while the existence of IgE in fluids at mucosal surfaces appears established, whether or not this is due to selective transport has not been established.

Plasma IgE transport to mucosal fluids has not been measured in nematode-infected rodents, despite the well-recognized elevated serum IgE response (13–16, 19). We therefore approached the question of how IgE might function in the gut by quantitatively defining its behavior after intravenous injection in T. spiralis–infected rats. Analysis of the distribution of ^{125}I-IgE myeloma protein showed a reproducible pattern of localization of whole-body counts in that active parasitic infection of the gut led to elevated ^{125}I-IgE counts in the intestine. In >50% of these experiments, lung counts were also elevated. Usually, counts associated with the small intestine tissue (the site of residence of T. spiralis) were elevated three- to fivefold over normal rats. Large-intestine counts were typically elevated by twofold or so lower, absolutely, than small-intestine counts. Small-intestinal wash fluids from infected rats contained high counts, and these could be as much as 10–15-fold higher than the comparable level of normal rats.

It was important to determine whether luminal counts represented degraded IgE or intact, high molecular weight IgE. Since, in rats, the liver is important in IgA transport (66), we determined the contribution of bile transport to total luminal counts. At the time of killing, the last 2.5-h collection of bile contained 283 counts, less than half of which would remain in the lumen at the point of killing because the transit time in the small intestine is 45–60 min (∼142 cpm). This is a negligible contribution to the overall recovery in the wash fluid 24 h after ^{125}I-IgE injection, which ranged between 8,000 and 28,000 cpm, depending on the amount of ^{125}I-IgE injected and the day of infection. Furthermore, the counts in bile fluid were all low molecular weight, and no high molecular weight material was detectable by PAGE analysis in bile samples, although it was evident in the gut lumen wash. An average of ∼20–25% of intestinal wash counts were due to high molecular weight IgE in infected rats. At 24 h, between 0.3 and 0.8% of total injected IgE was recoverable as intact IgE (mean 0.7%). With a small-intestine transit time of 45 min, over a 24-h period, ∼22% of the injected dose of IgE (24 h × 0.7% ÷ 0.75 h) will have entered the lumen of the gut. This assumes that the transport of IgE remains constant throughout this period. In reality, at 24 h, ∼45% of total initial counts have been lost in the urine and feces. Transport into the intestinal lumen is thus likely to be less at this point than earlier, when high ^{125}I-IgE levels are present in plasma. The estimate of 22% of the injected dose indicates that a substantial fraction of plasma IgE is likely to have entered the intestine. Since considerable local synthesis of IgE in the gut wall is taking place concurrently in infected rats (Richards, E. M., C. H. Wang, and R. G. Bell, unpublished results), it seems reasonable to expect that exogenous ^{125}I-IgE would have to compete with endogenously produced IgE for access to gut transport mechanisms.

The data further suggest that it is unlikely that intestinal transport was due to local damage or inflammatory processes. T. spiralis infection produces local damage to enterocytes (67), hyperemia (68), acute inflammation (69), and lymphocyte invasion (68) at the site of infection, albeit in temporally distinct sequences. Despite these underlying changes, ^{125}I-IgG1 failed to appear in intestinal wall tissues or intestinal wash fluids at any higher rate in infected rats than in control uninfected rat tissues. This indicates processes that are selective for IgE rather than simple plasma leakage. Since IgG1 is smaller

(mol wt = 155,000) than IgE (mol wt = 190,000), we would have expected 20–30% more IgG1 than IgE in the intestinal lumen if inflammation-related diffusion were the main determinant of immunoglobulin movement. In fact, the levels we found for IgG1 were usually <20% of the value observed for IgE. Presumably, uptake of IgE by the gut tissues reflects the appearance of IgE receptors on as yet undefined cell types.

We did not attempt to determine the actual site of intestinal transport of IgE except to show that bile transport was unimportant. IgE in the gut lumen of infected rats was the same molecular weight ($\sim$190,000) in nondenaturing gels as that injected, indicating that luminal IgE was not bound to a receptor. Both CD23 (70) and $\epsilon\beta$P (71) have been identified in gut epithelial cells, and either could be involved in uptake or transport processes. We found little evidence to support a role for FcϵRI in any intestinal cell population. Perhaps most FcϵRI receptors were already occupied but, due to the high affinity of binding, they are unlikely to be involved in transport. Expression of CD23 on enterocytes is increased in patients with enteropathies (70). While IEL possessed receptors for IgE that could be blocked by anti-CD23 antibody, it seems anatomically unlikely that IEL could transport IgE into the gut lumen. Our techniques for demonstrating CD23 on enterocytes by in vitro binding were compromised by the large amounts of mucus produced by goblet cells in this site during parasite infection.

Intestinal uptake of transferred ^{125}I-IgE was at least partially an inducible property of specifically immune lymphocytes. Previous work from this laboratory has documented the protective functions of CD4$^+$ OX22$^-$ cells isolated from the thoracic duct of rats infected 3 d previously with *T. spiralis* (19, 23, 33, 34, 41). These cells can transfer two distinct forms of anti–*T. spiralis* immunity, rejection of adult worms (33), and rapid expulsion (19). Their presence is an essential prerequisite for the passive transfer of rapid expulsion with specific IgE (19), but their precise role in this has not been defined. Adoptively transferred CD4$^+$ OX22$^-$ cells do not stimulate an intestinal mastocytosis, but rather an eosinocytosis (34). This raises the very interesting possibility that cells other than mast cells or even eosinophils may be important in the IgE-

dependent rapid expulsion process. The induction of IgE uptake by the nonparasitized gut after cell transfer further supports the view that intestinal binding of IgE is not a consequence of parasite-induced local damage or inflammation. The experiment with IL-4 further demonstrates that IgE binding in the gut is a process that can be influenced by a single cytokine and thus constitutes a significant new function for IL-4. While a lack of rat reagents hampers a more comprehensive examination of the role of IL-4 or that of other potential contributors (e.g., IL-10), the effect was nevertheless striking.

While the details of inducible intestinal IgE uptake and transport remain to be defined, we suggest that both processes open new avenues for the exploration of the role of IgE in protection against helminth infections and in allergic disease. The likelihood that comparable processes operate in lung was suggested by several of our experiments, which showed elevated uptake of intravenously transfused ^{125}I-IgE in lung tissue. If confirmed, this is significant for allergic disorders of the respiratory tract. Furthermore, the indication that IEL, a granulated cytotoxic cell population, have inducible IgE receptors, CD23 in this case, suggests new functions for this cell population. Finally, the amount of IgE transported from the plasma into the gut after its appropriate stimulation represents a substantial proportion of the total amount of IgE injected. This suggests that the gut, when stimulated, plays a minor role in determining the overall IgE balance sheet. In the introduction, the association between parasitic infections in humans and the low incidence of allergic disease in tropical countries was discussed. The data presented here indicate that intestinal nematode infections induce high activity in a gut-specific IgE transport process concurrently with infection, and that this is influenced by IL-4, the cytokine principally involved in upregulating IgE production. Intestinal nematode infections are ubiquitous in humans and other mammalian populations in natural settings. This suggests that the physiology and metabolism of IgE may be very different under conditions of chronic intestinal parasitism than it is in Western Europe and North America, where parasitic infections of the gut are rare but allergic disease is common.

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