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ORAL TOLERANCE TO DEXTRIN MEDIATED BY SPECIFIC SUPPRESSOR T-CELLS INDUCED IN THE INTESTINAL INTRAEPITHELIUM AND THEIR SYSTEMIC MIGRATION

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ABSTRACT

Antigens presented to the immune system through the oral route induce antigen specific secretory IgA and systemic unresponsiveness, termed oral tolerance (OT). We studied the induction of OT towards a diet antigen: dextrin (DEX) in rats that underwent protein deprivation and were further re-fed. Peyer's patches (PP), mesenteric lymph nodes (MLN) and spleen (Sp) cells from protein re-fed (R) rats mediated hyporesponsiveness after transfer into naïve recipient rats. Low numbers of MLN T cells transferred hyporesponsiveness while higher numbers transferred an enhancement of the delayed type hypersensitivity (DTH) reaction. MLN T cells were further separated based on their ability to bind Vicia villosa (VV). *MLN VV^- T cells, mainly $CD8^+$, mediated hyporesponsiveness and MLN VV^+ T cells ($CD45RC^+$ $CD4^-$ $CD8^-$ cells) abrogated the hyporesponsiveness. Moreover, Sp DEX adherent T cells were mainly $CD8^+$. Intestinal intraepithelial lymphocytes (iIELs) mainly $CD8\alpha^+$ $\gamma\delta$-TCR^+ cells also inhibited the DTH response to DEX after transfer. The positive DTH response to another carbohydrate (levan) indicates the specificity of the suppression to dextrin. Therefore, our data indicate that after oral administration of DEX, two different populations of T cells were generated: one found only in the MLN that mediated DTH responses and the other one capable of migrating from the intestinal intraepithelium through PP and MLN to the Sp, mediating systemic tolerance.*

Mucosal surfaces are continually challenged by pathogens, allergens, environmental toxins and food antigens, which enter the host through the oral route. The mucosal immune system is then triggered, mounting rapid local effector responses characterized by the presence of antigen specific secretory IgA in the secretions. Moreover, the specific immune response to fed antigens is also characterized by antigen specific systemic unresponsiveness called oral tolerance (1-3). Oral tolerance was first described at the beginning of the 20th century as the inability to induce systemic anaphylaxis in guinea pigs previously fed hen's egg protein (4). There has been extensive research in this field during the last three decades using protein antigens – mainly ovalbumin – and red cells (5-7). More recent studies have used the possibility to induce oral tolerance for the treatment of autoimmune disease in both animals and humans (8-12). Strong evidence indicated that after oral administration of a protein antigen, CD8 T cells capable of transferring oral tolerance into naïve recipients were induced (5,6,13,14). Nevertheless, it has also been shown that the induction of oral

tolerance was abolished by *in vivo* depletion of CD4 cells, but not CD8 cells, before and at the time of a single oral feed (15). Moreover, the possibility of abrogating oral tolerance has also been described, with the participation of CD8 cells, in this phenomenon (16). Therefore, the exact mechanism of oral tolerance induction, if only one, is not yet fully understood.

It is well known that malnutrition, and especially protein-deficiency provokes an impairment in secretory antibody responses and in cell mediated immunity that will be more or less severe depending on the degree of the deficiency and on the host's stage of development (17,18). We have studied how severe protein deficiency at weaning impairs IgA B cell differentiation at the level of Peyer's patches (PP), mesenteric lymph nodes (MLN) and the intestinal lamina propria, observing that not only the number of B cells but also the number of T cells decreased in protein-deficient rats (19-23). When the animals were adequately re-fed with protein, IgA B cell differentiation was reinitiated although control levels of IgA B cells were not reached. Moreover, in the MLN, the number of CD4 cells increased but did not reach the control values (24). Furthermore, immunohistochemical studies have shown an increase in the number of $\gamma\delta$-TCR$^+$ cells in the gut lamina propria and in the number of intestinal intraepithelial lymphocytes (iIELs) expressing CD8$\alpha\alpha$ CD25 and $\gamma\delta$-TCR (25). These findings were further confirmed by flow cytometric analysis showing higher numbers of CD8$^+$ $\gamma\delta$-TCR$^+$ cells in the re-fed animals (26). Protein-re-fed rats also exhibited a diminished delayed-type hypersensitivity (DTH) response to dextrin, a polysaccharide and the major ingredient in the diet (24), suggesting that oral tolerance to a polysaccharide had been induced. Hence, the aim of the present report was to study the mechanism by which hyporesponsiveness to dextrin had been established as well as to define and characterize the cells involved in the onset of this type of oral tolerance.

MATERIALS AND METHODS

Animals and Diets

Weanling male Wistar rats (21-23 days of age), from the closed colony bred at the Animal Facility of the Faculty of Pharmacy and Biochemistry at the University of Buenos Aires, were kept in the experimental unit at the Department of Food Science and Experimental Nutrition. Feeding the animals with a protein-free diet for 13-15 days induced protein deficiency in weanling rats. Animals that lost about 25% of the initial body weight were housed individually receiving a 20% casein diet for 21 days (protein re-fed: R). Animals that did not lose 25% of their initial weight were discarded. Mortality was always less than 10% for the complete experimental period. Control rats and normal (naïve) recipient rats received conventional diet in a pelleted form (Cargill, Buenos Aires, Argentina). The protein-free diet contained dextrin, vitamins, minerals and essential fatty acids. The 20% casein diet contained: casein (20%) dextrin, vitamins, minerals and essential fatty acids. Both diets were prepared as described previously (27).

Cells Suspensions

Spleen (Sp), MLN, and all PP were removed, placed in RPMI 1640 (Gibco, Grand Island, NY) plus 10% fetal calf serum (Gibco) (RPMI/FCS). PP, MLN and Sp were minced with scissors before pressing through a nylon sieve in the above medium. Sp were treated with ammonium chloride (0.83%) in order to lyse red cells. All cell suspensions were filtered through nylon wool before use to eliminate dead cells and debris. Cell recovery and viability was determined by trypan blue exclusion using a haemocytometer counting chamber (28). MLN and Sp pure T cells were obtained by using nylon wool columns (29). T cell purity was tested by an indirect immunofluorescence assay to determine the percentage of contaminating B cells in the

cell suspension. B cell contamination was always less than 5%.

Intestinal Intraepithelial Lymphocyte (iIELS) Isolation

iIELs were isolated as described before (26). Briefly, intestines were flushed with calcium- and magnesium-free Hank's supplemented with 0.035% sodium bicarbonate, PP were excised and the small intestine was first cut longitudinally and cut further into smaller fragments. These fragments were incubated in citrate buffer (pH 7.2) supplemented with 1mM dithiothreitol for 5 minutes at room temperature to remove mucus, rinsed thoroughly with Ca/Mg free Hank's, and incubated for 15 more minutes in RPMI/2% FCS. Supernatants were passed through nylon wool to remove debris. Cell suspensions were finally purified through Percoll (Pharmacia, Uppsala, Sweden) gradients.

Biomagnetic Cell Separation

Isolated iIELs were incubated with mouse monoclonal antibodies directed against the rat T cell subsets (SeraLab, Accurate Chemicals, Westbury, NY and Pharmingen, San Diego, CA) that we wanted to remove. Then, cells were incubated with Dynabeads M450 coated with goat anti mouse IgG (Dynal AS, Oslo, Norway). Dynabeads conjugated cells were retained by a magnetic field while unlabeled cells remained in suspension. This methodology allowed us to isolate the cells that expressed the desired phenotype very quickly and with a consistent high purity and viability (30,31).

Cell transfer experiments and DTH responses

PP or MLN or Sp cells from experimental donor rats (R rats) were intravenously transferred into naïve recipient rats (60 days old rats chow fed); the recipient rats were sensitized 24 hours later with the corresponding antigen: dextrin, casein or ovalbumin. Six days later, recipients were challenged into their hind footpads with the specific antigen. DTH was assessed by the degree of footpad swelling after challenge.

Total and Dynabeads purified populations of iIELs (CD8αα, CD4, αß-TCR, γδ-TCR, and CD8αα γδ-TCR) were transferred into naïve recipients as described above.

Casein and ovalbumin

Casein (CAS) and ovalbumin (OVA, Sigma, Saint Louis, MO) solutions were prepared as described elsewhere (32). Briefly, rats were sensitized subcutaneously by injecting 50 µg of the antigen emulsified in 40 µl of complete Freund's adjuvant (Difco, Detroit, MI). DTH was elicited by challenging with 30 µl of a 2% suspension of heat aggregated antigen in saline.

Dextrin and Levan

Rats were sensitized by intraperitoneal injection of 10 µg of dextrin or levan in saline (33,34). DTH was elicited by challenge with 7.5 µg of dextrin or levan in 30 ml of saline (31,35).

Phenotype of Transferred T Cells

The phenotype of MLN and Sp T cell populations was assessed by indirect immunofluorescence staining, using the following monoclonal antibodies: W3/25 (anti rat CD4), MRCOX8 (anti rat CD8) and MRCOX22 (anti rat CD45RC) from Seralab (36,37) as primary antibodies and a FITC conjugated goat anti mouse IgG (Cappel, division of Organon Teknika, Durham, NC) as the secondary antibody. Cell cytospins were read under an Olympus epiillumination fluorescent microscope. iIEL T cell phenotype was determined after Dynabeads purification using indirect immunofluorescence staining and the following monoclonal antibodies anti rat: CD8α, CD8ß, γδ-TCR, αß-TCR, CD4 from Pharmingen (San Diego, CA) as

TABLE 1
DTH Response to Dextrin Transfer of PP, MLN and Sp Cells from Donor R Rats into Naïve Recipient Rats

Donor rats	Source of cells	Number of cells	DTH response to: DEX[a]	CAS[a]
Control	PP	$0.30x10^6$	0.41±0.07(3)	0.42±0.08(5)
R	PP	$1.20x10^6$	0.19±0.05(9)[c]	0.56±0.07(10)
Control	MLN	$0.70x10^7$	0.51±0.04(4)	0.33±0.03(2)
R	MLN	$3.50x10^6$	0.23±0.03(18)[d]	0.31±0.05(13)
Control	Sp	$0.80x10^7$	0.47±0.03(4)	0.52±0.06(4)
R	Sp	$3.50x10^7$	0.18±0.02(17)[d]	0.38±0.05(16)
None	None[b]	—	0.42±0.06(13)	0.42±0.11(5)

PP, MLN and Sp cells from Control (normal) or donor R rats were transferred i.v. into naïve recipient rats; 24 hrs later the recipients were immunized with DEX (i.p.) or CAS (s.c.). Six days later recipients were challenged in their hind footpads. [a]X±SE: increase in footpad thickness 24 hrs after challenge, expressed in milimeters; (n): number of rats. [b]controls that did not receive cells. [c]$p<0.02$, [d]$p<0.005$; values significantly different from rats receiving cells from control donors.

primary antibodies followed by the FITC conjugated goat $F(ab)_2$ fragment to mouse IgG (Cappel).

Dextrin Adherent T Cells

Dextrin adherent T cells were purified from spleen T cells following a modification of the panning technique using dextrin coated Petri dishes (38). Briefly, polystyrene Petri dishes were incubated with dextrin in saline (1 mg/ml), overnight, at 4°C. Non-bound antigen was removed by rinsing plates with saline (0.15M), and Hank's balanced salt solution (HBSS). Spleen cells were suspended in HBSS and their concentration was adjusted to 1.5-2.0 x 10^7 cells/ml. Dextrin coated Petri dishes were incubated with cells for 1 hr at room temperature (RT). Non-bound (dextrin non-adherent) cells were removed and the Petri dishes were rinsed three times with HBSS at RT. Then, chilled HBSS was added to the plates, and they were incubated for 20 minutes at 4°C. Dextrin adherent cells were removed by vigorous pipetting. Plates were washed again with chilled HBSS, and the remaining cells were also collected.

Vicia Villosa Adherent T Cells

Vicia villosa adherent (VV^+) T cells were prepared using MLN pure T cells according to a protocol described elsewhere (39). Briefly, *Vicia villosa* (Sigma) dissolved in citrate saline buffer, pH 5.5, was applied to 10cm tissue culture grade Petri dishes and incubated for 45 min at RT. The Petri dishes were washed three times with saline, then incubated with RPMI plus 2% FCS for 15 min at RT. MLN T cells were adjusted to 7.5 x 10^6 cells/ml in RPMI plus 5% FCS. Ten ml of the cell suspension was added per coated Petri dish and incubated for 45 min at 37°C. Non-adherent cells were gently removed (VV^-). Residual non-adherent cells were

TABLE 2
Induction of a DTH Response to Dextrin After Transfer of Purified T Cells from Donor R Rats

Donor rats	Source of cells	Number of cells	DTH response to: DEX	CAS	OVA
R	MLN	$5.5x10^5$	0.17±0.07(5)[a]	0.61±0.31(4)	0.68±0.20(5)
R	Sp	$1.5x10^7$	0.16±0.05(9)[a]	0.50±0.14(3)	0.52±0.17(6)
None	None	—	0.61±0.09(6)	0.61±0.07(4)	0.64±0.14(6)

MLN and Sp pure T cells from donor R rats were transferred i.v. into naïve recipient rats; 24 hrs later the recipients were immunized with DEX (i.p.), CAS (s.c.) or OVA (s.c.) and 6 days later recipients were challenged in their hind footpads. Pure T cells were prepared as described in Materials and Methods. [a]$p<0.002$ significantly different from the control that did not receive cells. X±SE (n).

removed after a gentle wash with RPMI 1640. The bound cells were removed from the plate by adding 5 ml N-acetyl-D-galactosamine (Sigma), 1 mg/ml in saline. The plates were incubated for 5 min at 37°C and then cells were vigorously pipetted with a Pasteur pipette.

Statistical Analysis

Statistical analysis was performed using ANOVA followed by Tukey-Kramer or Student's *t* test.

RESULTS

Transfer of PP, MLN and Sp Cells from R into Naive Recipients

PP, MLN and Sp cells from donor R rats transferred into naïve recipient rats inhibited the DTH response to dextrin, but not to casein. These results suggested that cells from R were capable of eliciting inhibition of a DTH response to a fed antigen *(Table 1)*.

Transfer of Sp Purified T Cells

Sp T cells from donor R rats when transferred into naïve recipient rats were able to inhibit the DTH response to dextrin, but not to casein or ovalbumin *(Table 2)*. These results suggested that R rats elicited an antigen specific mechanism. Purified Sp T cells were separated according to their ability to bind dextrin into dextrin adherent (DEX^+) T cells and dextrin non-adherent (DEX^-) T cells. DEX^+ Sp T cells were responsible for the inhibition of a DTH response to dextrin after transfer into naïve recipients *(Table 3)*. DEX^- T cells did not inhibit the DTH response after transfer. The distribution of $CD4^+$ cells in both cell preparations was similar but DEX^+ cells comprised a higher proportion of $CD8^+$ cells suggesting that these cells could play an important role in the inhibition of a DTH response.

Transfer of MLN Purified T Cells

MLN T cells from donor R rats when transferred into naïve recipient rats were able to inhibit the DTH response to dextrin, but not to casein or ovalbumin *(Table 2)*. The finding that transfer of as little as 5.5×10^5 purified MLN T cells could inhibit the DTH response to dextrin led us to study if this hyporesponsiveness was dependent on the

TABLE 3
Phenotypic Characterization of Dextrin Specific Sp T Cells from Donor R Rats Responsible for the Inhibition of a DTH Response to DEX after Transfer into Naïve Recipient Rats

Spleen T Cells	Number of cells	DTH response to DEX[a]	Phenotype CD4	CD8
Total T	$1.5x10^7$	0.20±0.06(8)[b]		
T DEX^+	$2.1X10^6$	0.20±0.04(6)[b]	64.0±6.2	47.0±4.0[c]
T DEX^-	$2.4x10^7$	0.47±0.10(6)	60.0±3.0	18.0±4.0
None	—	0.42±0.04(4)		

Cells from donor R were transferred i.v. into naïve recipient rats, 24 hrs later the recipients were immunized with DEX (i.p.) and 6 days later recipients were challenged in their hind footpads. Pure T cells, DEX^+ and DEX^- T cells were prepared as described in Materials and Methods. [a]Percentage of positive cells, data from two separate experiments pooled together X±SE (n). [b]$p<0.02$ significantly different from the control that did not receive cells; [c]$p<0.02$ DEX^+ vs DEX^-.

TABLE 4
The Induction of a Positive DTH Response to Dextrin Depends on the Number of MLN T Cells Transferred from R Rats

Number of cells	DTH response to DEX
$5.5x10^5$	0.17±0.07(5)[a]
$1.8x10^6$	0.44±0.13(4)
$1.0x10^7$	0.64±0.09(4)[b]
—	0.44±0.06(7)

MLN T cells from donor R rats were transferred i.v. into naïve recipient rats; 24 hrs later the recipients were immunized with DEX (i.p.) and 6 days later recipients were challenged in their hind footpads. [a]$p<0.01$, [b]$p<0.05$ significantly different from the control that did not receive cells.

number of cells transferred *(Table 2)*. However, when 1.8 x 10^6 purified MLN T cells were transferred into naïve recipients, the induction of a DTH reaction to dextrin was observed in recipient rats *(Table 4)*. Moreover, 1.0 x 10^7 MLN T cells increased the DTH reaction in the recipient rats as compared with control rats *(Table 4)*. It is worth noticing that transferring increasing numbers of donor cells abrogated unresponsiveness. Was this effect due to the existence of up-regulatory cells within the MLN T cell population? In order to answer this question and taking into account that an up-regulatory population has been recognized by their ability to bind *Vicia villosa* (VV) (39), we separated MLN T cells from donor R rats into VV^+ and VV^- MLN T cells before transferring them into naïve recipients. The data presented in Table 5 indicated that VV^- MLN T cells inhibit the appearance of a DTH reaction to dextrin in naïve recipient rats. However, VV^+ MLN T cells allowed for the development of a DTH response. The phenotypical analysis of VV^+ and VV^- T cell populations showed significant differences. VV^- cells mainly comprised $CD8^+$ cells; meanwhile VV^+ cells comprised $CD45RC^+$ and $CD4^-$ $CD8^-$ cells.

TABLE 5
Effect of VV^+ and VV^- MLN T Cells from Donor R Rats on the Induction of a DTH Response to Dextrin after Transfer into Naïve Recipient Rats

MLN T cells	Number of cells	DTH response to DEX	Phenotypic characterization[a]		
			CD4	CD8	CD45RC
Total T	$2.45x10^5$	$0.11\pm0.03(6)^b$			
T VV^-	$2.12x10^6$	$0.10\pm0.02(6)^b$	23.5±5.5	43.0±5.0	26.5±1.5
T VV^+	$2.85x10^6$	0.37±0.06(6)	15.0±4.0	14.0 ± 2.0^c	49.5 ± 4.5^c
None	—	0.35±0.04(11)			

Cells from donor R rats were transferred i.v. into naïve recipient rats; 24 hrs later the recipients were immunized with DEX (i.p.) and 6 days later recipients were challenged in their hind footpads. VV^+ and VV^- MLN T cells were prepared as described in Materials and Methods. [a]Percentage of positive cells, data from two separate experiments pooled together X±SE. [b]$p<0.001$ significantly different from the control that did not receive cells. [c]$p<0.05$ VV^+ vs VV^-.

Transfer of Intestinal IELs

Taking into account that there is no information available in the literature as to the possible anatomical site for the induction of T cells that specifically recognize polysaccharides, we decided to study the iIELs since these cells were in close contact with the antigen. Furthermore, the ability of iIELs to migrate out of the intestinal compartment has been demonstrated (40). First, we observed that transfer of iIELs from R rats inhibited the DTH response to dextrin in naïve recipients *(Fig. 1A)*. Then, we decided to separate iIELs using Dynabeads in order to define the cell phenotype associated with inhibition of a DTH response to dextrin. We observed that $CD8\alpha^+$, $\gamma\delta$-TCR^+ and to a lesser extent $CD4^+$ cells could inhibit the DTH response in the recipients *(Fig. 1B and 1C)*. Moreover, we decided to isolate the $CD8\alpha^+$ $\gamma\delta$-TCR^+ cells in order to ascertain that this was the population responsible for the inhibition of the DTH response in the recipients *(Fig. 1D)*.

DTH Response to Levan

To further define the specificity of the reaction, R rats and controls were i.p. immunized either with dextrin or levan and the DTH response was measured 6 days later. Dextrin-fed rats inhibited the DTH response to dextrin but were unable to inhibit the response to levan *(Fig. 1E)*.

DISCUSSION

Numerous studies have shown that continuous feeding of soluble proteins or particulate antigens results in the induction of T helper cell dependent IgA responses at mucosal sites, concurrent with oral tolerance (11,13). On the contrary, there is limited information about the induction of oral tolerance towards complex carbohydrates. Nevertheless, it has been shown that polysaccharide antigens such as dextran B1355S can also induce IgA type responses and oral tolerance (1,41,42). In the present study, we used dextrin, which is a polysaccharide of intermediate chain length

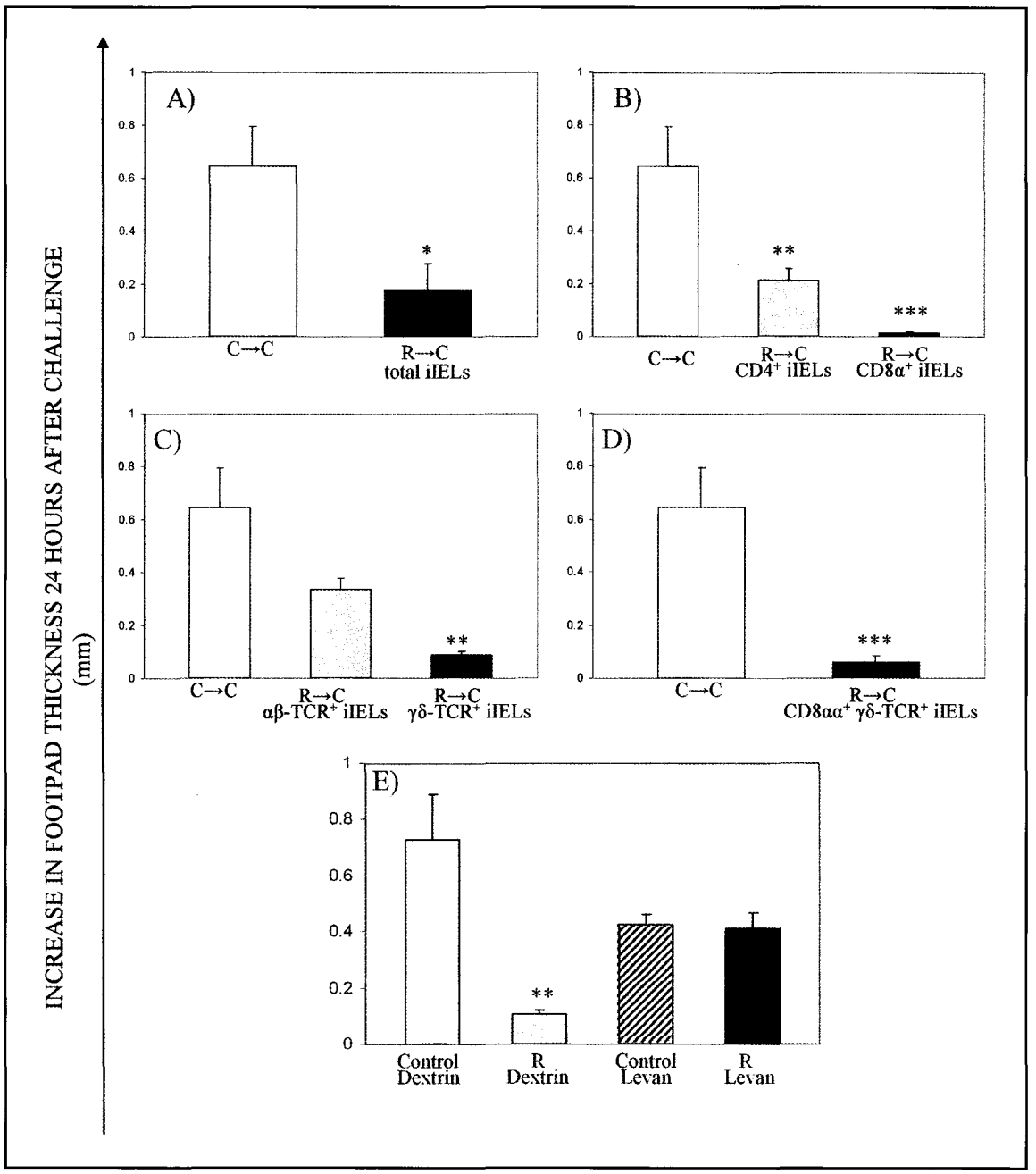

*Fig. 1. Transfer of iIELs into naïve recipients. Total iIELs, CD4+, CD8α+, αß-TCR+, γδ-TCR+ and CD8αα+ γδ-TCR+ cells from R rats were transferred i.v. into naïve recipients that were immunized i.p. 24 hours after transfer, and challenged in their hind footpads 6 days after transfer. Four to six recipient rats received 1x10^6 cells. Cells were prepared as described in Materials and Methods. Data are presented as X±SE, and represent the increase in footpad thickness 24 hrs after challenge, data are expressed in millimeters. Significant differences between recipients receiving iIELs from PR rats and recipients receiving unseparated iIELs from normal rats are shown, *p< 0.02; **p<0.01; ***p<0.001 (A to D). Control and PR rats were immunized i.p. with dextrin or levan, and their DTH response was measured 6 days later (E).*

formed from starch components by the action of amylases. Its α-D-glucopyranoside linkages are α (1,4) and α (1,6) and differ from dextran B1355S and dextran B512 whose linkages are α (1,3) and α (1,6) (43). Dextrin was the source of carbohydrates in the experimental diet. Rats received approximately 6 grams of dextrin per day.

We have previously demonstrated that R rats were unable to mount a DTH response towards dextrin (24). The antigen specificity of the response has now been proved by immunizing R rats with another carbohydrate, levan, a polymer of ß-D fructose *(Fig. 1E)* (34). R rats responded to levan as control rats. In this paper, we studied whether hyporesponsiveness to dextrin was associated with anergy or with active suppression. PP, MLN or Sp cells from R rats were transferred into naïve recipients that were immunized 24 hrs after transfer and challenged 6 days later. Cells from R rats inhibited the DTH response to dextrin after transfer *(Table 1)*. These results confirmed that hyporesponsiveness to dextrin was associated with suppression of DTH responses. The cells involved in the induction of hyporesponsiveness to dextrin were DEX adherent, mainly $CD8^+$ T cells *(Tables 2 and 3)*. Therefore, our data implied that oral tolerance to dextrin was induced and was mediated by DEX specific $CD8^+$ T cells. These data also suggested that specific T cells could have been induced by dextrin in the PP, migrated to MLN and then to the systemic lymphoid organs, such as the spleen. The above results were in agreement with those obtained by others when feeding sheep red blood cells, ovalbumin and myelin basic protein (5,6,14).

Some results obtained upon transfer of MLN cells proved to be very complex to interpret since we observed that only a low number of MLN donor T cells from R rats were able to inhibit the dextrin specific DTH response after transfer. Higher numbers of MLN donor T cells were capable of inducing a higher dextrin specific DTH response after transfer *(Table 4)*. The existence of functional up-regulatory T cells that may be induced in the PP after continuous exposure to a T-dependent antigen and act in the presence of suppressor T cells abrogating their effect has been described (46-49). These PP up-regulatory T cells selectively abrogated T cell mediated suppression of the IgA isotype responses (50). They were characterized in mice and humans by their ability to bind the lectin *Vicia villosa* (39,51). It could be speculated that the MLN T cell population that favored DTH responses could comprise either antigen-specific helper T cells for DTH responses or antigen specific up-regulatory T cells. Thence, MLN T cells were separated into VV^+ and VV^- cells and transferred into naïve recipients. On the one hand, the finding of a VV^- T cell population in MLN, that mediated the suppression of the DTH response to dextrin in recipient rats was not surprising, and in agreement with work done by others using spleen and MLN cells (5,6,13,14). On the other hand, the finding within the MLN T cell population of VV^+ cells, that upon transfer into recipient rats allowed the induction of a DTH response to dextrin, has not been previously reported. The VV^+ T cell population included a high percentage of cells bearing the CD45RC marker *(Table 5)*. Moreover, its phenotype partially corresponded to the population termed Th1 in rats: rat CD45RC is equivalent to mouse CD45RB (37,52,53). Nonetheless, other authors have shown that after repeated feeding of large doses (500 mg) of a protein antigen to transgenic mice, antigen specific T cells in the PP express the activation marker CD44 with low or no CD45RB and undergo apoptosis (9). In our experimental model, the presence of this unique population suggested that oral tolerance could potentially be abrogated under special circumstances, like protein malnutrition, allowing for the induction of DTH responses (54).

Elegant experiments using a model of oral infection with *Toxoplasma gondii* have demonstrated that antigen specific iIELs were primed and afterwards migrated to PP and

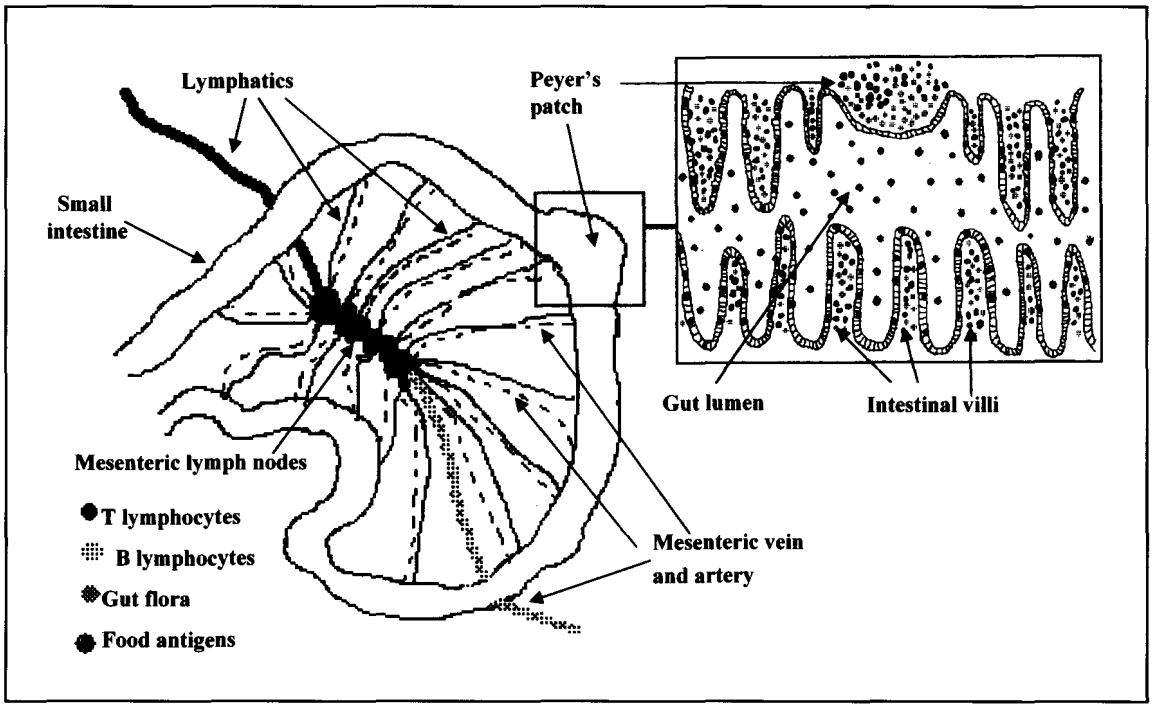

Fig. 2. The inductive site for oral tolerance to dextrin. The small intestine is irrigated by the mesenteric artery that bifurcates into small arterioles that reach the intestinal wall. From the intestinal wall small venules and lymphatics exit the gut towards the mesenteric lymph nodes. The small intestine has specialized areas as the Peyer's patches where protein antigens from the gut lumen are presented to T and B lymphocytes. Other food or bacterial antigens are absorbed through the epithelial cell layer in the intestinal villi by a not well known mechanism. T cells primed in the intestinal intraepithelium could either migrate to PP and then to MLN or migrate directly to MLN. Antigens can also reach the MLN. Some T cells will proliferate and be retained in the MLN whereas others will exit MLN through lymphatics and reach the general circulation becoming memory cells or migrate to effector sites.

MLN (40). In the present study we used dextrin, which is part of the daily diet, and thence, it is also in close contact with iIELs. Taking into account these data, we considered the hypothesis whether dextrin specific iIELs would be primed in our experimental model and whether these cells would inhibit the DTH response against dextrin and migrate to PP, MLN and Sp. Our experiments showed that iIELs could also specifically inhibit a DTH response against DEX. $CD8\alpha^+$ $\gamma\delta$-TCR^+ cells were the main iIEL population responsible for the inhibition observed in recipient rats *(Fig. 1)*. Therefore, our results suggested that dextrin specific DTH inhibitory cells have been induced in the intestinal intraepithelium and migrated to PP, MLN and Sp.

It has been shown that intestinal epithelial cells from 2,4-dinitrochlorobenzene-fed mice inhibited hapten-specific T cell activation *in vitro* (44). Taken together, these data and our own results, it could be postulated that intestinal epithelial cells could act as antigen presenting cells for the induction of oral tolerance against polysaccharides or haptens. Oral tolerance to protein antigens could be induced through different mechanisms since the most recent data suggested that MLN was the critical induction site for high-dose oral tolerance in the absence of PP (45). A schematic drawing showing the possible inductive sites for oral tolerance to proteins and polysaccharides is presented in *Fig. 2.*

Therefore, we propose that in the presence of dextrin or other food antigens, specific suppressor T cells for DTH responses can be induced in the intestinal intraepithelium and migrate to PP, MLN and Sp. Suppressor cells would follow this migration pathway in order to suppress systemic DTH responses to foodborne antigens, as described for other antigens (1,5,6,10,13). Nevertheless, under special pathological conditions like secondary immunodeficiency provoked by severe protein deficiency, oral tolerance could be overridden, and either helper T cells or up-regulatory T cells could appear. These cells could be induced either in the PP or in the intestinal intraepithelium, as in other models (50), and then migrate to the MLN where we found them. Importantly, in our model these VV^+ cells induced a DTH response against DEX only in the recipient rat but did not migrate to the spleen in the donor animals. Therefore, we can conclude that in rats that underwent severe protein deficiency and re-feeding, the appearance of an antigen specific suppressor T cell population in the intestinal intraepithelium, the site of antigen contact, was favored. This population migrated through PP, MLN and reached the Sp, suggesting a systemic distribution of cells that inhibited DTH responses to fed antigens. Another T cell population that favored DTH responses was demonstrated. Nonetheless, these cells seemed to be retained in MLN, since donor rats did not show signs of local DTH responses, and their activity was only demonstrated upon transfer.

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