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Antigen presentation by epithelial cells of the rat small intestine

I. KINETICS, ANTIGEN SPECIFICITY AND BLOCKING BY ANTI-Ia ANTISERA

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SUMMARY

Columnar epithelial cells (EC), isolated from the proximal small intestine of the rat, bind ovalbumin (OVA) by a non-specific, cold-inhibitable mechanism and continue to express Ia antigens after 24 hr culture *in vitro*. Lymph node T cells from rats immunized with OVA proliferate following 18 hr coculture with EC and OVA. This accessory cell function of EC is antigen-specific and is blocked by anti-Ia monoclonal antisera.

INTRODUCTION

The mechanisms governing the immunogenic or tolerogenic consequences of antigen feeding are complex: the dose and molecular conformation of antigen (Vives, Parks & Weigle, 1980), the age of the individual (Hanson, 1981), and its genetic constitution (Stokes, Swarbrick & Soothill, 1983) have all been shown to be mechanistic factors.

Peyer's patches (PP) are generally thought to be the foci of interactions between food and bacterial antigens and the mucosal immune system. Antigen presentation (Richman, Graeff & Strober, 1981a) and the induction (Spalding *et al.*, 1984), maintenance (Green *et al.*, 1982) and suppression (Ngan & Kind, 1978; Richman *et al.*, 1981b) of immune responses have all been demonstrated *in vitro*, using PP-derived cells. However, surgical removal of PP does not ablate mucosal antibody responses (Hamilton *et al.*, 1981), and although antigens are absorbed across the normal villous epithelium of the small intestine, the role of the epithelium in the induction and regulation of mucosal immune responsiveness has not been studied.

Columnar absorptive cells of the small intestinal villous epithelium have been shown to express class II antigens of the major histocompatibility complex (MHC) in the mouse (Parr & McKensie, 1979), the rat (Mayrhofer, Pugh & Barclay, 1983) and man (Scott *et al.*, 1980). In the rat, epithelial cell (EC) MHC class II (Ia) antigens are not a product of bone marrow-derived cells (Mayrhofer *et al.*, 1983) and are, therefore, presumed to be synthesized within EC. In the normal adult rat their expression is restricted to fully differentiated columnar EC on the upper two-thirds of the villus (Mayrhofer *et al.*, 1983). They are not normally expressed on immature crypt cells, and large bowel epithelia are, under normal circumstances, Ia-negative (personal observation). However, in experimentally induced intestinal inflammation (Barclay & Mason, 1982) or graft-versus-host disease (Mason, Dallman & Barclay, 1981; Barclay & Mason, 1982) crypt cells may be induced to express Ia antigens. Similarly, Ia-negative epithelial cell lines can be induced to express Ia antigens in culture with a Con A-induced intraepithelial lymphocyte (IEL) or spleen cell product (Cerf-Bensussan *et al.*, 1984). Further, EC in inflamed regions of large bowel mucosa in patients with inflammatory bowel disease have been shown to express HLA-DR antigens (Selby *et al.*, 1983). These observations indicate that the expression of EC class II antigens can be altered in immune processes and suggest that they may participate in immune reactions in the gut mucosa.

MHC class II antigens are integral membrane glycoproteins that act as restriction elements controlling the presentation of antigen to immunoregulatory T cells. Their restricted distribution within the gut epithelium—only on those EC involved in nutrient transport from the gut lumen—raises the possibility that Ia-bearing EC may function as antigen-presenting cells, thereby activating immunoregulatory T cells directly at the mucosal portal of antigen entry. We have investigated this using Ia antigen-bearing EC isolated from rat small intestine.

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Abbreviations: EC, epithelial cell(s); IEL, intraepithelial lymphocyte(s); IL-1, interleukin-1; IL-2, interleukin-2; HGG, human gamma globulin; MHC, major histocompatibility complex; Mø, macrophage(s); OVA, ovalbumin; PP, Peyer's patch(es).

MATERIALS AND METHODS

Animals

Male WF/Hsd Br rats were obtained from Harlan Sprague–Dawley, Indianapolis, IN, and were used at 7–10 weeks of age. Rats were maintained under constant environmental conditions on Purina Rodent Chow and water *ad libitum* throughout the study.

Antigens and immunizations
Chicken egg albumin (OVA), fraction V (Sigma Chemical Co., St Louis, MO) and human gamma globulin (HGG), fraction II (Miles Laboratories Inc., Naperville, IL) were used without further purification. For immunization, antigens were dissolved in PBS, emulsified with Freund's complete adjuvant (Sigma Chemical Co.) and 400 μl of emulsion containing 100 μg of antigen were injected into the footpads of each rat. Ovalbumin for use in uptake studies was iodinated with carrier-free ^{125}I by the method of Hunter & Greenwood (1962).

Media
Hanks' balanced salt solution (HBSS, without calcium or magnesium) and RPMI-1640 were obtained from Gibco Laboratories Inc., Grand Island, NY. RPMI was supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 units/ml penicillin, 100 μg/ml streptomycin, 40 μg/ml gentamicin, 20 mM HEPES and, except where stated, with 10% fetal calf serum (FCS, Hazleton Dutchland Inc., Denver, PA).

Cell isolation
Epithelial cells. Epithelial cells were isolated from non-primed rats. The proximal 10 cm of small intestine, comprising duodenum and proximal jejunum, was excised, flushed with 50 ml cold PBS, everted and ligatured at each end to form an everted sac. The sac was washed extensively in HBSS and incubated in HBSS containing 0·5 mM EDTA at room temperature for 5–10 min with constant stirring to remove the villous epithelium. Cell sheets and clumps were disrupted by gentle aspiration using a Pasteur pipette. The single-cell suspension was then washed twice in RPMI, counted in a haemocytometer and included immediately in assays.

T cells. The popliteal and brachial lymph nodes draining the footpads were removed 8–15 days after immunization. The nodes were teased and passed through an 80-mesh stainless steel sieve. The resultant cell suspension was rigorously depleted of adherent cells by passing over two successive nylon-wool columns using the method of Julius, Simpson & Herzenberg (1973).

Spleen cells. Spleen cells were prepared by teasing and sieving spleens from unimmunized rats. Erythrocytes were removed by hypotonic lysis with sterile distilled water. Adherent cells were prepared by incubation of spleen cells in Falcon tissue culture dishes (Becton Dickinson, Oxnard, CA) for 2 hr at 37° and were recovered by incubation on ice for 30 min, followed by vigorous pipetting. Resultant cells were morphologically $57 \pm 4\%$ macrophages (Mø), $50 \pm 7\%$ MRC OX-6$^+$ and are hereafter referred to as splenic Mø. They were irradiated (2000 rads) using a caesium source prior to inclusion in proliferation assays.

Interleukin preparation
Interleukin-1 (IL-1) was prepared as the supernatant from splenic Mø (10^6/ml) incubated at 37° with 1 μg/ml muramyl dipeptide (Calbiochem, San Diego, CA) for 24 hr. Interleukin-2 (IL-2) was prepared by incubation of unfractionated spleen cells (5×10^6/ml) in RPMI containing 2 μg/ml concanavalin A (Pharmacia, Uppsala, Sweden) for 24 hr at 37°. The supernatant was collected and delectinized by the method of Rich & Pierce (1974). IL-1- and IL-2-containing supernatants were dialysed against RPMI, filter-sterilized and stored at −20° until use.

Proliferation assays
Assays were carried out in 96-well, flat-bottomed microtitre plates (A/S Nunc, Roskilde, Denmark). Responder wells each received 2×10^5 T cells and other components as indicated in the text in a total volume of 200 μl per well. Plates were incubated for 96 hr in a humidified atmosphere of 95% air, 5% CO_2 at 37°. Four hours before termination of cultures, 1 μCi [^{3}H]TdR (5·0 Ci/m mole, Amersham Corp., Arlington Heights, IL) was added to each well. Plates were harvested using a semi-automatic harvester (MASH II, M. A. Bioproducts, Walkersville, MD) and filter pads were counted for radioactivity in ACS scintillation fluid (Amersham Corp.).

Monoclonal antibodies
The monoclonal antibodies used in the study were as follows: W3/25, MRC OX-8, MRC OX-6, MRC OX-17 and W6/32. All were obtained from Accurate Chemical Corp., Westbury, NY.

Immunofluorescence
Cell suspensions were incubated for 30 min on ice with the optimum dilution of mouse anti-rat monoclonal antibody, washed three times with cold PBS and incubated for 30 min on ice with FITC-conjugated rabbit anti-mouse immunoglobulins (DAKO Corp., Santa Barbara, CA) absorbed with 10% heat-inactivated WF/Hsd BR serum. Cells were washed a further three times in cold PBS, mounted in PBS + 40% glycerol and viewed under epi-illumination in a fluorescence microscope. Photographs were taken on Kodak Tri-X film.

In vitro *uptake of ^{125}I OVA by isolated EC*
Five-ml aliquots of EC at 2×10^6/ml were incubated on ice or at 37° with 10^{-8} M ^{125}I-OVA in serum-free RPMI or RPMI supplemented with 10% FCS. One-ml samples were removed at Time 0 and thereafter at various time intervals. Samples were immediately placed on ice, centrifuged at 250 *g* for 2 min and the uptake of ^{125}I was determined following differential counting of supernatant and pellet in a screened tube using the method of Chantot & Saul (1978). Binding was determined in the presence and absence of a large excess (5×10^{-6} M) of unlabelled OVA to determine total and non-specific association of labelled protein with cells.

Analysis of data
For each experiment, pooled EC isolated from two to four unprimed rats and pooled lymph node T cells from four to six immunized rats were used. Each experiment was performed at least twice. In proliferation assays, statistical significance between groups did not differ in replicate experiments. However, values of c.p.m. for comparable groups of replicate experiments varied. For this reason, the results given in each case are from a single representative experiment. Analysis of variance was applied to quadruplicate values to compare each variable of each group, and statistical significance of differences was calculated using the Bonferroni test.

RESULTS

Characterization of isolated epithelial cells

The pinocytotic activity of isolated EC precluded reliable assessment of their viability by conventional dye exclusion

assays. Instead, vital cell functions related to antigen presentation were investigated. The presentation of antigen to T cells by Mø is dependent on the expression of Ia molecules on the Mø plasma membrane (Lee, Wilkinson & Wong, 1979), and also on the ability of Mø to bind and 'process' the antigen (Ziegler & Unanue, 1981). Therefore, as a preliminary to investigating the accessory cell capabilities of EC, the maintenance of antigen-binding capacity and of Ia antigen expression were assessed as an indication of the effect of the isolation procedure on these parameters.

Ia antigen expression. Immediately after the EC isolation procedure, 60–76% (mean 68%) of the total isolated cells were morphologically columnar and expressed cytoplasmic and plasma membrane Ia antigens, shown by indirect immunofluorescence staining with MRC OX-6. After 24 hr culture at 37°, the proportion of EC retaining Ia expression in the cell isolate had dropped to 36% (range 30–41%).

Cytoplasmic staining with MRC OX-6 (Fig. 1) was granular and concentrated in the supranuclear region. Membrane staining was both linear and granular and was confined to the basolateral plasma membranes, with only very occasional faint staining of the apical microvillus surface. Identical staining characteristics were observed using the antibody MRC OX-17 (anti-I-E), although intensity of staining was always less than with MRC OX-6. Rounded crypt cells in the isolates were unstained.

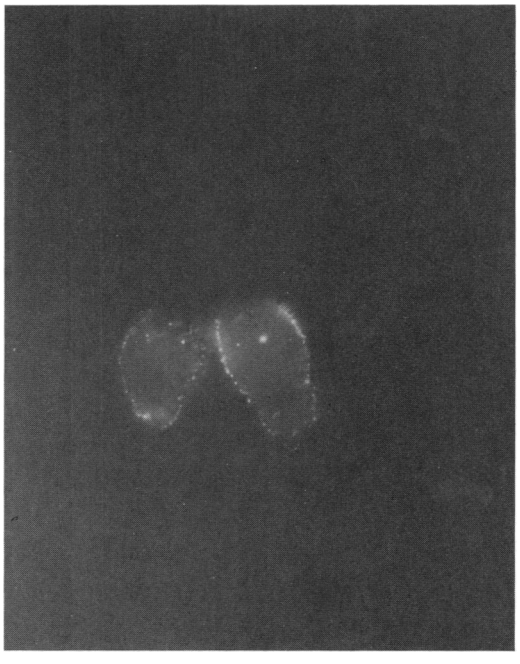

Figure. 1. Epithelial cells isolated from the proximal small intestine of normal WF rats and stained by immunofluorescence for Ia antigens with monoclonal antiserum MRC OX-6 (anti-I-A). Granular cytoplasmic staining is confined to apical, supranuclear regions, while membrane staining is limited to basolateral membranes. Apical microvilli (top) are unstained. Magnification × 300.

Epithelial cell isolates typically also included 2–4% MRC OX-8$^+$ IEL, a variable number of which were granulated. W3/25$^+$ cells were absent from isolates and MRC OX-6$^+$ Mø were not observed.

Antigen uptake. Binding of ^{125}I-OVA to EC at 37° reached a maximum within 30 min and was maintained at this level over the 2 hr incubation period (Fig. 2). However, a substantial component of this binding was non-specific, i.e. it was not displaced by excess unlabelled OVA. Binding of ^{125}I-OVA in RPMI supplemented with 10% FCS did not differ from non-specific binding in serum-free RPMI. Moreover, ^{125}I-OVA bound to EC in the presence of FCS was not displaced by excess unlabelled OVA. Binding was not detectable in incubations carried out at 4°.

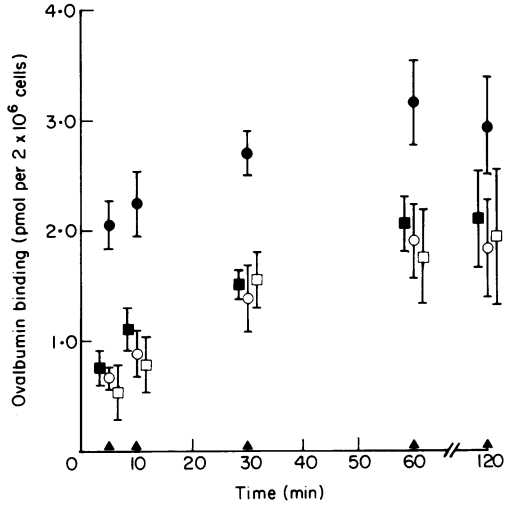

Figure 2. Binding of ^{125}I-ovalbumin by epithelial cells (EC) isolated from the rat small intestine. In serum-free RPMI: total binding at 37° (●); binding at 37° in the presence of excess unlabelled OVA (○); total binding at 4° (▲). In RPMI + 10% FCS: total binding at 37° (■); binding at 37° in the presence of excess unlabelled OVA (□). Mean ± SD of three experiments.

The results are consistent with the interpretation that binding of OVA to isolated EC does not require an OVA-specific receptor, displacement taking place to the same degree in the presence of unrelated serum proteins as with excess unlabelled OVA. Total inhibition of binding at 4° may indicate the participation of cold-inhibitable endocytotic mechanisms, suggesting that the 'binding' seen at 37° represents uptake of labelled OVA into the cell after attachment to a non-specific protein-binding site.

Antigen presentation by epithelial cells

Continuous 96-hr cultures. Splenic Mø provided efficient accessory cell function to OVA-induced T-cell proliferation (Table 1). The ratio of T cells to Mø giving maximum T-cell proliferation was variable between experiments. In contrast, T cells cultured with EC showed no increase in [^{3}H]TdR incorporation above background levels. In fact, in most cultures containing EC, the [^{3}H]TdR incorporation into T cells was significantly lower than background.

The proliferative response of T cells to antigen is thought to be dependent upon release of the T-cell product IL-2, which is in turn mediated by IL-1 synthesized by the antigen-presenting cell (Mizel & Ben-Zvi, 1980). However, addition of exogenous IL-1

Table 1. Comparative accessory cell function of macrophages and epithelial cells in ovalbumin-driven T-cell proliferation

Treatment	Cell ratio T cell:accessory cell	Proliferative response* (c.p.m. [³H]TdR incorporation) Mø	EC
None	1:0	586	
OVA	1:0	808	
OVA+IL-1	1:0	1010	
None	2·5:1	4839	175
OVA	10:1	41,325	414
OVA	5:1	73,583	266
OVA	2·5:1	60,305	299
OVA+IL-1	10:1	50,287	473
OVA+IL-1	5:1	70,638	309
OVA+IL-1	2·5:1	67,550	631

* 2×10^5 T cells from OVA-immunized rats were cultured for 96 hr in quadruplicate wells with graded doses of Mø or EC with 100 μg/ml OVA and IL-1 (25%) as shown. Data are expressed as mean c.p.m.

to cultures showed that the absence of EC accessory cell function in direct proliferation assays could not be attributed to deficient synthesis by EC of this cytokine.

Discontinuous cultures. Further analysis showed that EC in prolonged culture exerted an inhibitory effect on the proliferation of cocultured T cells (Bland & Warren, 1986). In order to overcome this inhibitory influence, T cells were physically separated from EC following an induction coculture in the presence of antigen. Specifically, T cells were incubated either alone or with EC and/or OVA in 35 mm diameter plastic tissue culture dishes (10^7 T cells/dish) for 18 hr at 37°, whereupon cells were removed from the dishes (viable T-cell recovery from dishes was 94–100% of starting population, mean 96%), layered on Ficoll–Hypaque gradients (density 1·080 g/ml) and centrifuged at 400 *g* for 20 min. Efficiency of T-cell recovery from the gradient interface was $77 \pm 4\%$ ($n=12$) of T-cell numbers originally added to dishes, with $<1\%$ contaminating EC. Preliminary experiments showed that separation of T cells from EC in this way did not significantly affect T-cell subset ($W3/25^+$ *vs* MRC $OX\text{-}8^+$) proportions. After fractionation, T cells were washed, plated out and cultured for up to 120 hr at 37°.

Figure 3a shows the proliferative responses of T cells treated in this way. Only T cells precultured with EC and OVA demonstrated significant proliferation, with a maximum response at 96 hr. T cells precultured either alone or with EC or OVA independently did not show subsequent proliferation. Addition of IL-2 (25%) throughout the secondary cultures caused amplification of the proliferation shown by T cells precultured with EC and OVA (Fig. 3b). The increased proliferative response by T cells precultured with OVA alone presumably reflects antigen presentation during the 18 hr preculture by residual Mø within the T-cell population.

Kinetics of antigen presentation by EC

In order to analyse the optimum length of the initial (antigen presentation) cultures, i.e. the minimum time required for

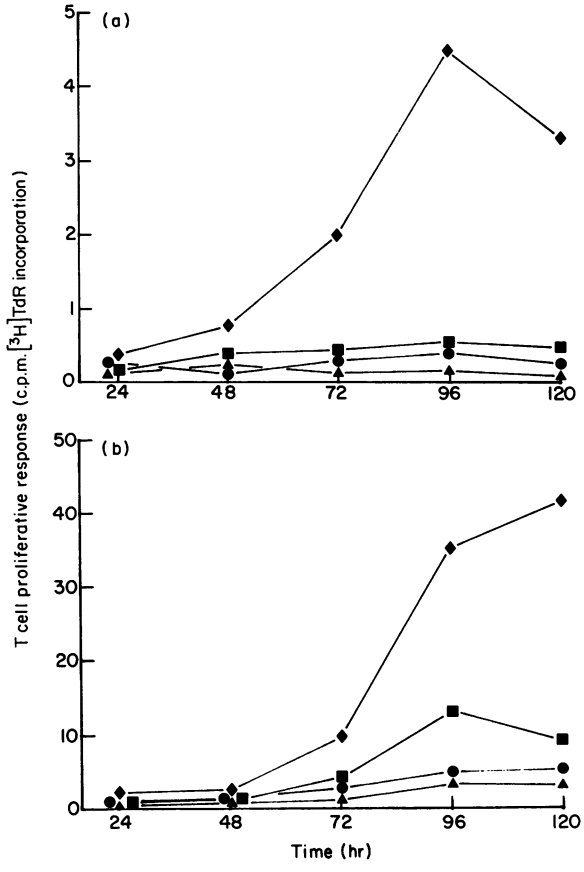

Figure 3. Proliferative response of immune T cells after ovalbumin (OVA) presentation by intestinal epithelial cells (EC). T cells were cultured alone (▲); with EC only (●); with OVA only (■); or with EC+OVA (♦) for 18 hr, isolated over Ficoll–Hypaque and allowed to proliferate in (a) RPMI or (b) RPMI + 25% IL-2 for the times indicated. Points represent the mean c.p.m. from quadruplicate wells. Standard errors were $<10\%$.

antigen presentation to take place and to exclude depression of T-cell proliferation by EC inhibitory factors, T cells were precultured with EC and OVA over a range of culture periods from 1 to 18 hr. T cells were then isolated from these cultures and cultured alone for 96 hr. Figure 4 shows that, although significant proliferation took place following shorter precultures, 18 hr preculture induced the maximum proliferative response. A preculture period of 18 hr was, therefore, used throughout the remainder of the study.

Effect of cell ratio on antigen presentation

T cells were precultured for 18 hr with OVA and increasing doses of EC to determine the T:EC ratio optimum for antigen presentation. T cells isolated from precultures comprising 10:1 and 5:1 ratios showed significant proliferation compared with control cultures (Fig. 5). There was no significant difference between the proliferative responses of T cells from 5:1 cultures or 10:1 cultures. T cells from precultures comprising T:EC of 2·5:1 did not proliferate significantly. Thereafter, T:EC ratios of 5:1 were used in induction cultures.

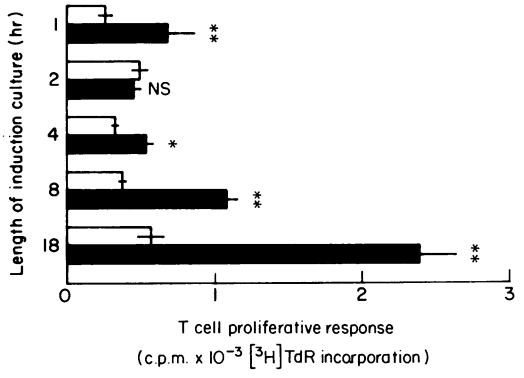

Figure 4. Time-course of antigen presentation by intestinal epithelial cells (EC). T cells were cultured with ovalbumin (OVA) alone (▭), or with EC + OVA (■), for the times indicated, isolated over Ficoll–Hypaque and allowed to proliferate for 96 hr. Results represent the mean c.p.m. ± SEM of quadruplicate wells. Significantly greater than OVA only group at $P<0.05$ (*) or $P<0.01$ (**). NS, not significant.

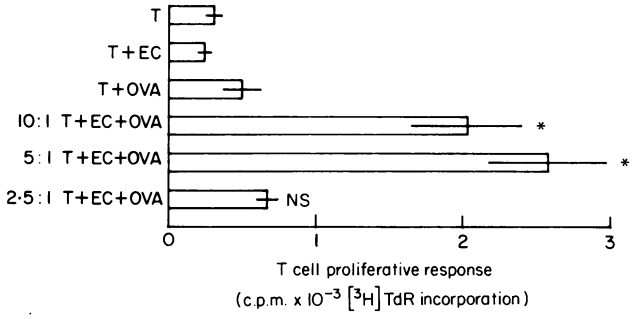

Figure 5. Effect of T cell:epithelial cell (EC) ratio on antigen presentation by intestinal EC. T cells were cultured with ovalbumin (OVA) and graded doses of EC for 18 hr, isolated over Ficoll–Hypaque and allowed to proliferate for 96 hr. Results represent the mean c.p.m. ± SEM from quadruplicate wells. Significantly greater than proliferation of T cells cultured with OVA only, $P<0.05$ (*). NS, not significant.

Antigen specificity of antigen presentation by EC

T cells from rats immunized with either OVA or HGG were cultured for 18 hr with various combinations of EC and OVA or HGG. T cells precultured with EC in the presence of the appropriate antigen subsequently demonstrated significant proliferation compared to controls (Fig. 6). T cells precultured with the inappropriate antigen did not proliferate significantly. Although T cells from rats immunized with HGG proliferated following exposure to EC and HGG, indicating effective presentation of HGG by EC, the proliferative response was always less than that of OVA-primed T cells following presentation of OVA by EC.

Effect of anti-Ia antisera

Induction cultures were established with T cells, EC and OVA in the presence or absence of monoclonal antisera against Ia antigens of I-A (MRC OX-6) or I-E (MRC OX-17) specificities. The mouse anti-human MHC class I antigen monoclonal antibody W6/32 (which does not recognize rat determinants)

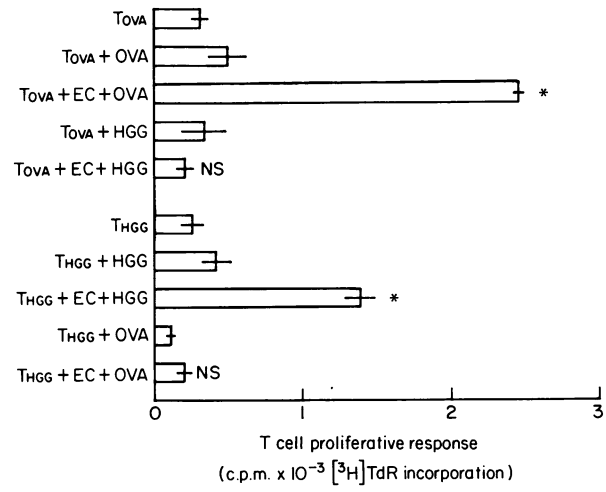

Figure 6. Antigen specificity of antigen presentation by intestinal epithelial cells (EC). T cells from rats immunized with ovalbumin (OVA, upper), or with human gamma globulin (HGG, lower) were cultured with EC and OVA or HGG for 18 hr, isolated on Ficoll–Hypaque, and allowed to proliferate for 96 hr. Results represent the mean c.p.m ± SEM from quadruplicate wells. Significantly greater than proliferation of T cells cultured with OVA or HGG alone, $P<0.001$ (*). NS, not significant.

was used as a control. Preliminary investigations showed that significant blocking of antigen presentation was achieved by MRC OX-6 or MRC OX-17 at final dilutions as low as 1 in 4000. However, the greatest dilution showing consistent blocking was 1 in 2000, and this dilution was used for all three antisera in the experiment depicted in Fig. 7. Significant inhibition of antigen presentation was observed for both anti-Ia antisera, although neither antiserum reduced subsequent T-cell proliferation down to control levels. No significant difference was observed between the blocking capacity of MRC OX-6 or MRC OX-17. Addition of the control antiserum W6/32 to induction cultures did not affect subsequent T-cell proliferation, i.e. it did not interfere with antigen presentation.

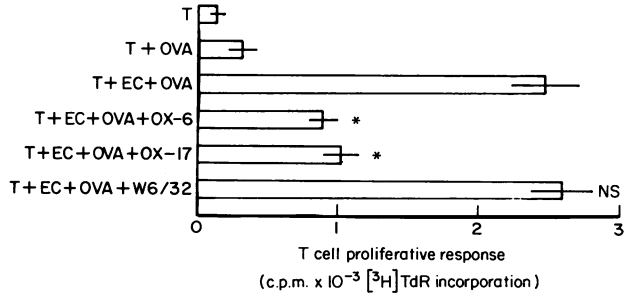

Figure 7. Effect of anti-Ia antisera on antigen presentation by intestinal epithelial cells (EC). T cells were cultured with EC and OVA and MRC OX-6 (anti-I-A), MRC OX-17 (anti-I-E), or W6/32 (anti-human HLA-A,B,C) antisera for 18 hr, isolated on Ficoll–Hypaque and allowed to proliferate for 96 hr. Results represent the mean ± SEM from quadruplicate wells. Significantly less than proliferation of T cells cultured with EC and OVA in the absence of antisera, $P<0.05$ (*). NS, not significant.

DISCUSSION

A satisfactory method for the isolation of Ia$^+$ columnar EC in high purity is described. A possible criticism of the use of isolated EC in these types of experiments is that fully differentiated absorptive EC are non-dividing cells and so are incapable of sustained growth *in vitro*. In addition, these cells show distinct polarity *in situ*, and it is possible that loss of this polarity during isolation from the parent tissue may result in inhibition of polarity-dependent functions. However, preliminary experiments showed that isolated EC maintained protein-binding function. Also, more than 50% of isolated cells continued to express Ia antigens after 24-hr culture. Thus, the ability to bind antigen and to maintain Ia expression, probably the two most essential criteria regarding antigen-presenting cells, were both met by isolated EC in our experiments. The use of established epithelial cell lines as putative antigen-presenting cells would overcome possible problems associated with EC viability in culture but, in our view, such cells would represent an unacceptable departure from the *in vivo* EC type. These cell lines must, perforce, be established from crypt cells capable of cell division. It is unlikely that the expression of Ia antigens (Cerf-Bensussan *et al.*, 1984) or the ability to process antigen shown by the progeny of such cells would parallel those parameters in villous columnar cells.

Initial experiments coculturing T cells and EC for 96 hr suggested that EC were ineffective as antigen-presenting cells. However, in separate investigations (Bland & Warren, 1986) we have shown that isolated EC release a variety of factors, including a non-dialysable factor which inhibits the proliferation of cocultured T cells. Physical removal of T cells from the suppressive influence of EC after the initial 18 hr coculture with antigen revealed the capacity for EC to mediate antigen-specific, Ia antigen-dependent antigen presentation.

T-cell proliferation induced by EC-mediated antigen presentation, although statistically significant and of consistent amplitude, was less than that induced by splenic Mø. Efficiency of antigen presentation may be controlled by a variety of factors, including (i) quantitative (Janeway *et al.*, 1984) or qualitative (Cullen *et al.*, 1981) variations in Ia antigen expression by the antigen-presenting cell, (ii) differences in antigen processing, (iii) the requirement for cytokines synthesized by antigen-presenting cell and responding T cell, and (iv) by a variety of potentiator mechanisms operating in association with the T-cell membrane receptors for antigen and Ia.

Comparative determinations of Ia antigen density on EC and splenic Mø have not been made, but we have found that similar dilutions of anti-Ia antisera mediate blocking of OVA presentation by both types of presenting cell (unpublished observations). This suggests that both cell types express similar levels of Ia antigen, functionally relevant in antigen presentation. Successful presentation of protein antigen requires that some degree of antigen 'processing' takes place prior to recognition by the T-cell receptor. This probably involves proteolytic cleavage of the protein, revealing epitopes reactive with the T-cell receptor (Shimonkevitz *et al.*, 1983). Degradation of proteins certainly takes place during absorption from the gut lumen (Stern & Walker, 1984), but the relative contributions of intraluminal proteases, microvillous peptidases and intracellular lysosomal hydrolases to such 'processing' have not been determined. Moreover, the immunological relevance of gut-processed antigen is uncertain. Nothing is known of antigen processing by isolated EC. Antigen presentation by Mø has been shown to require the participation of Mø-derived IL-1 (Mizel & Ben-Zvi, 1980) which, in turn, induces the expression of IL-2 receptors on responding T cells (Kaye *et al.*, 1984). We have recently identified a factor with the functional characteristics of IL-1 which is released by isolated EC in short-term culture (Bland & Warren, 1986). The enhanced responsiveness of T cells to IL-2 following 18 hr culture with EC and OVA, seen in the present study, suggests an increased synthesis of T cell IL-2 receptors, possibly induced by this EC factor.

The demonstration of Ia antigen-restricted antigen presentation by intestinal EC lends support to the hypothesis (Bland, 1985) that the intestinal epithelium participates directly in the regulation of gut mucosal immunity. Briefly re-stated: antigens, absorbed on a selective basis, pass through EC in endosomes and form complexes with locally synthesized Ia antigens which are re-expressed by exocytosis on the basolateral plasma membrane. The antigen–Ia 'complex' is recognized by intraepithelial T-cell receptors, and these T cells migrate into the lamina propria and to extramucosal sites, mediating rapid immunoregulatory signals to update the immune response to absorbed antigen. We have evidence suggesting that antigen presentation by gut epithelial cells does result in the induction of immunoregulatory T cells (Bland & Warren, 1986), but although IEL are likely candidates as the responding cell *in vivo*, this remains to be confirmed.

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