The Accessory Cell Activity of Porcine Intestinal Macrophages in the Induction of T Cell Responses

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ABSTRACT. Although intestinal macrophages stimulated significant T cell responses to MHC class II antigens and polyclonalmitogens, they were weak stimulators of ovalbumin-driven responses. These cells showed numerous and large endocytic vacuoles, lysosomes and phagolysosomes which may be indicative of active phagocytosis during the isolation process. The poor antigen presenting activity of the cells in protein antigen-elicited may be an *in vitro* artifact attributed to acquired inefficiency in antigen uptake and/or processing.—KEY WORDS: antigen presenting cell, intestinal macrophage, MHC class II antigen, mixed leukocyte response, oxidative mitogenesis.

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Non-lymphoid accessory cells have been shown to be present in the intestinal lamina propria of the mouse, rat, guinea and man [7, 11, 15, 17, 18, 20, 21]. These cells have been shown to express major histocompatibility complex (MHC) class II molecules [7, 16, 19–21]. Thus, it is very possible that these cells may act as antigen presenting cells (APC) in the induction of appropriate mucosal immune responses.

Although a number of studies have been carried to investigate mucosal immune responses in a variety of animals, very few attempts have been done to evaluate the antigen presenting activity of potential APC which are present in the lamina propria and other related mucosal sites. Studies aimed at elucidating the accessory cell role of either macrophages of dendritic cells have been done only in the mouse and man. It has been shown that whereas intestinal lamina propria macrophages and dendritic cells of the mouse were suppressive and stimulatory respectively [13], human cells [10] were capable of inducing mixed leukocyte responses (MLR). Allogeneic MLR has thus been the only proliferation system which has been used to evaluate the accessory cell role of intestinal macrophages and/or dendritic cells. Therefore, the aim of this study was to evaluate the ability of porcine intestinal macrophages to induce ovalbumin-elicited responses and compare this with their efficacy in the induction of MLR and oxidative mitogenesis.

MATERIALS AND METHODS

Medium: Tissue culture medium consisted of RPMI 1640 (Gibco, Paisley, Scotland) supplemented with 10% heat inactivated foetal calf serum, 2 mML-glutamine (Gibco), 1 mM sodium pyruvate (Flow Laboratories, Irvine, Scotland), 20 mM Hepes (Flow Laboratories), 20 μg/m*l* gentamicin (Flow Laboratories), penicillin (100 units/m*l*)/streptomycin (100 μg/m*l*) and 10 mM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, U.S.A.).

Immunization: Weaner and growing pigs (2–6 months old) were immunized with 1 mg of ovalbumin (Sigma) emulsified in Freund's complete adjuvant (Difco Laboratories, Detroit, U.S.A.). Animals were injected in the neck and were boosted 3–4 times at an interval of 15 days with the same dose of ovalbumin in phosphate buffered saline. Prescapular lymph node cells were isolated 5–10 days after the last boost.

Isolation of intestinal lamina propria macrophages: Serosal-muscle layers were peeled from aseptically collected small intestines which were cut into small fragments. Lamina propria cells were isolated after sequential stirring of mucosal fragments six times for 1 hr each in ethylene diamine-tetra acetic acid followed by enzymatic digestion. Tissue fragments were then stirred in washing medium (tissue culture medium with only 5% foetal calf serum) containing 100 units/m*l* of collagenase type V and hyaluronidase type 1-S (Sigma) for 90 min. The procedure was repeated twice. Cell suspension from the last two stages of enzyme digestion were pooled together, resuspended in 5 m*l* of 5% Nycodenz (Nycomed AS, Oslo, Norway; density 1.15 g/m*l*), layered over 3 m*l* of 40% Nycodenz and centrifuged at 650 g for 20 min at room temperature. Cells at the interface were collected, washed three times in washing medium and cell viability was determined using trypan blue.

Isolation of T cells: Prescapular lymph node were aseptically collected from sacrificed animals, meshed through wire sieve and passaged twice on nylon wool columns [9]. Non-adherent cells were used as responder cells (T cells) in proliferative assays.

Oxidative mitogenesis: T cells were treated with 2.4 mM of sodium meta periodate (Sigma) in phosphate buffered saline using standard procedures [2, 3]. Treated cells were then used in oxidative mitogenic assays.

Proliferative assays: 2×10^5 ovalbumin-primed T

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cells/200 μl/well in tissue culture medium were cultivated in six replicates alone or with 500 μg/m*l* ovalbumin with or without intestinal macrophages at T cell: accessory cell ratios of 1:1, 10:1 and 100:1. Cells were cultured for 96 hr. In other assays, the same number of either allogeneic or sodium periodate-treated T cells were cultured alone or with APC at T cell: APC ratios of 1:1 and 10:1. Cells were cultivated for 120 and 72 hr for allogeneic MLR and oxidative mitogenesis respectively. Four hr before termination of cultures, cells were pulsed with tritiated thymidine (Amersham International PLC, Buckingamshire, UK, specific activity 5.0 Ci/mmol) at 1.0 μl/20 μl/well. Cells were then harvested onto glass fibre plates (Skatron AS, Lier, Norway) using a multi-harvester (Skatron AS) and radioactivity incorporation was measured using a liquid scintillation counter (LKB Wallac Rack Beta, Wallac Oy, Turku, Finland). Results were expressed as mean counts per minute (CPM) of six replicates with standard error of means (SEM). Data were statistically analyzed using multiple regression analysis of variance to find out differences between experiments and treatments. Where differences in treatments were observed, regression coefficient ratios were used to analyze statistical significant differences between the various treatments (ie between control and test cultures established to test whether intestinal macrophages can enhance T cell proliferation). In individual experiments the data were analyzed using unpaired Students' *t*-test.

Transmission electron microscopy: Pelleted intestinal macrophages or tissues were fixed in 2.5% glutaraldehyde (Sigma) in 0.1 M sodium cacodylate buffer before post-fixing in 0.1% osmium tetroxide in 0.2 M cacodylate buffer. Cells or tissues were dehydrated in 70, 90 and 100% alcohol each for 10 min; for 1 hr in propylene/Epon mixture and overnight in Epon. Pelleted cells or tissues were embedded in Epon and finally cut on a Reichert OMU2 ultramicrotome. Sections were then stained with 1% uranyl acetate and lead cytrate. Evaluation of the morphological features was carried out by transmission electron microscopy.

RESULTS

The results of assessment of the effectiveness of intestinal macrophages in eliciting ovalbumin-driven responses are shown in Table 1. It was observed that with exception of experiment 3, T cells cultured with ovalbumin did not induce significant responses. Proliferative levels in other control cultures (APC + antigen and APC + T cells) were not statistically different from those of other controls. With the exception of experiment 2, co-culturing T cells with intestinal macrophages in the presence of ovalbumin resulted in significant but weak enhancement of responses ($p<0.001$) when cells were cultured at a cell ratio of 1:1. Inconsistent results including either further enhancement or equal or decreased levels, were observed at a cell ratio of 10:1, whereas no enhancement was evident at a T cell: accessory cell ratio of 100:1. Thus, although no enhancement was evident in experiment 2, significant but inconsistent and weak responses were elicited by intestinal macrophages. Treatment of APC with 2 mM chloroquine (Sigma) for 3 hr during the antigen pulsing abrogated the responses (data not shown).

In contrast to the weak induction of T cell responses to ovalbumin, intestinal macrophages were capable of inducing significant ($p<0.001$) T cell proliferation in allogeneic MLR assays (Table 2). The responses were observed only at a T cell: APC ratio of 1:1, whereas no responses were elicited at a cell ratio of 10:1. The cells also induced significant ($p<0.001$) responses of sodium periodate-treated T cells (Table 3).

In order to elucidate this very weak antigen presenting activity, we evaluated the ultrastructural morphology of the cells by electron microscopy and related it to the morphology *in situ*. It was observed that the nuclei of

Table 2. Accessory cell activity of macrophages in allogeneic MLR[a)]

Experiment	T cells alone	APC alone	T cells+APC	
			1:1	10:1
1	123(27)	225(50)	7,449(1,158)	440(40)
2	362(73)	400(75)	4,079(765)	480(125)
3	150(15)	280(30)	7,358(1,575)	629(124)

a) Allogeneic T cells (2×10^5 cells/well) in six replicates were co-cultured alone or with intestinal macrophages at T cell: APC ratios of 1:1 and 10:1 for 120 hr.

Table 1. Accessory cell activity of intestinal macrophages in ovalbumin presentation[a)]

Experiment	T cells alone	T cells +antigen	T cells+APC +antigen at cell ratio of 1:1	T cells+APC +antigen at cell ratio of 10:1	APC +antigen	T cells +APC
1	105(44)	135(66)	1,386(97)	33,352(405)	123(29)	195(31)
2	207(27)	137(22)	529(99)	127(18)	105(18)	159(26)
3	227(66)	2,717(312)	4,498(523)	4,318(356)	128(30)	245(37)
4	467(67)	565(673)	1,838(208)	1,838(208)	173(33)	701(82)

a) T cells (2×10^5 cells/well) in six replicates were cultured alone or with ovalbumin or APC as control cultures. In test cultures T cells were cultured with antigen and APC for 96 hr.

Table 3. Accessory cell activity of macrophages in oxidative mitogenesis[a)]

Experiment	Untreated T cells	Treated T cells	Untreated T cells+APC	Treated T cells + APC
1	195(24)	177(23)	274(34)	3,416(441)
2	255(54)	12,047(1,028)	198(32)	48,513(5,132)
3	461(70)	602(133)	571(79)	2,127(182)
4	217(62)	568(93)	327(48)	4,459(574)
5	131(21)	180(59)	123(25)	14,282(1,589)

a) Periodate-treated T cells (2×10^5 cells/well) were co-cultured alone or with intestinal macrophages at a T cell: APC ratio of 1:1 for 72 hr.

isolated cells showed aggregates of chromatin and the cytoplasm contained numerous endocytic vacuoles/vesicles, lysosomes and phagolysosomes (Fig. 1a).

Endocytic vacuoles were of variable sizes. Cells *in situ* were irregular in shape, had fewer and small endocytic vesicles, lysosomes and phagolysosomes, and chromatin material was diffusely distributed around the periphery of the nuclei (Fig. 1b).

DISCUSSION

It was evident that intestinal macrophages enhanced T cell proliferation in response to ovalbumin but the responses were inconsistent and very weak. This was in contrast to the effectiveness of the cells in inducing allogeneic mixed leukocyte responses (MLR) and polyclonal mitogenesis. The effective induction of MLR and oxidative mitogenesis has indicated that porcine intestinal lamina propria macrophages express MHC class II glycoproteins and can provide other costimulatory factors essential for T cell proliferation. These costimulatory factors include interleukin-1 (1L-1). Although McKenzie *et al.* [12] have shown that human dendritic cells can induce MLR in the absence of IL-1, it is generally thought that costimulatory factors are essential in all T cell responses [8]. It can therefore be speculated that the poor accessory cell role of intestinal macrophages in ovalbumin-induced T cell responses is not due to deficiency in IL-1 secretion or lack of MHC class II glycoproteins but may be due to other costimulatory factors and/or adhesion molecules.

The presentation of ovalbumin requires its uptake and processing into a form recognizable by the T cell receptor. Poor uptake and processing of antigen can be a reason for the inefficiency of antigen presenting ability of potential

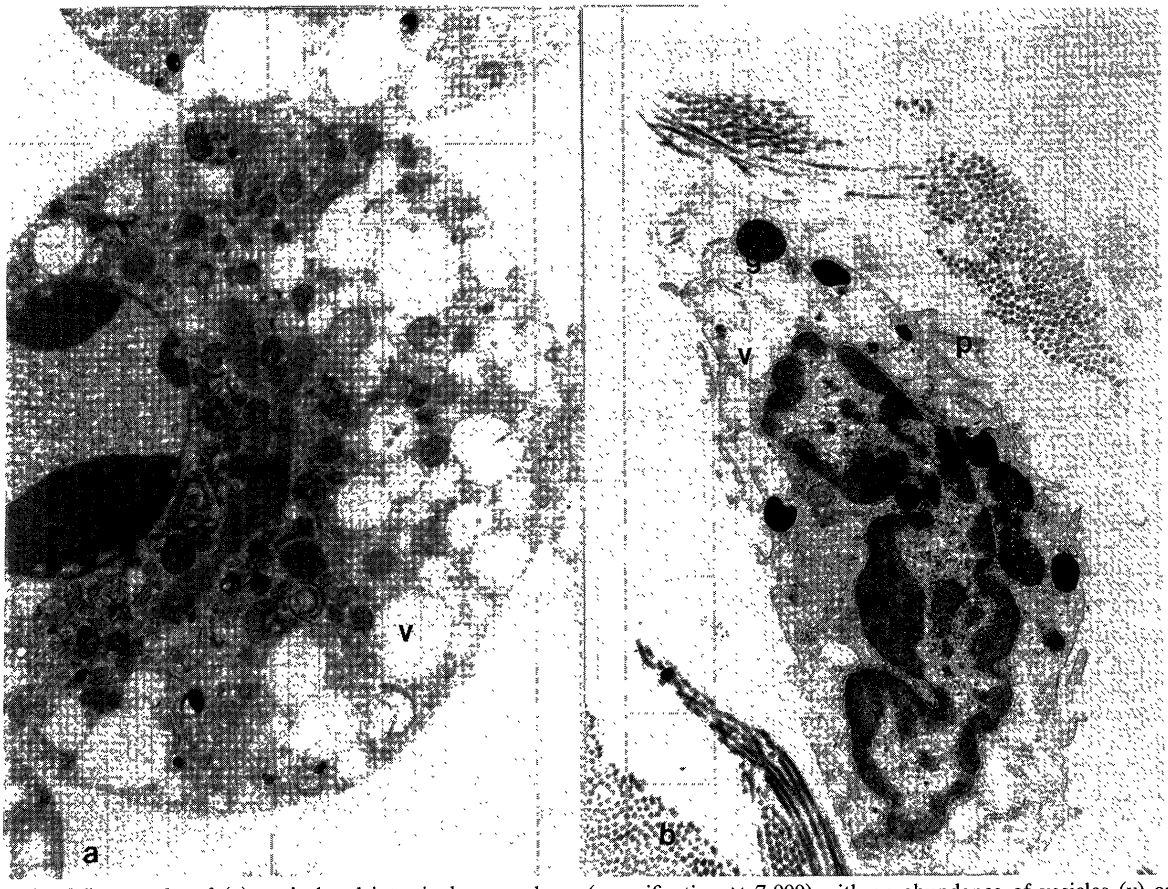

Fig. 1. Micrographs of (a) an isolated intestinal macrophage (magnification × 7,000) with an abundance of vesicles (v) and lysosomes (l) and (b) a cell *"in situ"* (magnification × 10,000) showing processes (p), vesicles (v) and phagolysosomes (g).

APC [4]. Thus, the capability to process antigens and the amount of MHC class II molecules may determine the number of antigenic peptide/MHC class II complexes available to T cell receptors. It was observed in this study that isolated cells had numerous endocytic vesicles, lysosomes and phagolysosomes. Possibly this is a reflection that these cells were actively phagocytosing or endocytozing fluids and other material during the isolation process. This apparent high endocytic activity during cell isolation process may result in depletion of lysosomal enzymes necessary for processing of antigens or cells may reach their ability to further endocytose thus resulting in reduced antigen uptake. In addition, mucus which has been shown to interfere with cell fractionation [14] was a major problem associated with isolation of intestinal cells. This occurred despite using mucolytic agents [1]. Coating of cells with mucus may also have reduced antigen uptake. However, this needs further investigation.

The different distribution pattern of chromatin in isolated cells compared to those *in situ* may be indicative of some damage which may interfere with the biosynthesis and re-cycling of MHC class II molecules which are responsible for rescuing antigens from total degradation [5]. Hence, this poor activity appears to be an *in vitro* artifact attributed to effects of isolation on the functional ability of cells isolated from the gastrointestinal tract by enzymes and mechanical forces [6].

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