Functional Interleukin-2 Receptors on Intestinal Epithelial Cells

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Abstract

The presence of receptors for the cytokine IL-2 was assessed in the IEC-6 cell line established from normal rat crypt epithelium and primary intestinal epithelial cells. ^{125}I-IL-2 was found to specifically bind to subconfluent IEC-6 cells. Maximal binding was observed within 30 min after addition of the ligand; binding could be inhibited by excess unlabeled IL-2 or addition of antibody to the IL-2 receptor. Both intermediate and low affinity receptors with approximate K_d of 10 and 100 pM, respectively were present. Kinetic analysis were consistent with the results of Western blot analysis using an antisera to the 75-kD IL-2 receptor β chain. IL-2 receptors appeared to be functional; addition of IL-2 led to modulation of proliferation with initial stimulation at 24 h followed by inhibition at 48 h. This effect could be blocked by addition of antibody to the IL-2 receptor β chain. IL-2 treatment could be shown to enhance expression (range = 4- to 50-fold stimulation) of TGF-β, as well as the lectin protein mac-2, in IEC-6 cells. The relevance of observations in the IEC-6 cell line to intestinal mucosa in vivo was supported by the demonstration of a gradient of expression of the IL-2 receptor in primary rat intestinal epithelial cells by Western blot analysis. In addition, mRNA for the IL-2 receptor-β chain was demonstrated by Northern blot analysis using mRNA from primary rat intestinal epithelial cells depleted of detectable contaminating intraepithelial lymphocytes by two cycles of fractionation on Percoll gradients. Collectively, these observations suggest that the range of cellular targets of the putative lymphokine IL-2 is broader than appreciated, and IL-2 may serve to integrate epithelial and lymphocyte responses in the intestinal mucosa. (*J. Clin. Invest.* 1993. 92:527–532.) Key words: cytokine • receptor • TGF-β_1 • mucosal immunity • lymphokine

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Introduction

Over the past decade, a continually expanding repertoire of proteins have been identified, which contribute to interactions among cellular components of the immune system, as well as other cell types. Various interleukins and other cytokines have been found to be widespread in their distribution and to exert protean effects among different cell populations (1–4). This complex network of protein factors may be especially important in the mucosa of the gastrointestinal tract. Indeed, many cytokines have been found to be produced by cells within the lamina propria (2–8). Conversely, cellular constituents of the lamina propria have been found to respond to various cytokines. More recently, a variety of reports suggest that the so-called cytokine network may encompass the epithelial cell population within gastrointestinal tract mucosa. Thus intestinal epithelial cell populations have been found to express and/or respond to several broadly active cytokines including IL-1, IL-6, TNF, IFN-γ, and TGF-β (2–10). It should be noted that these "shared" cytokines encompass proteins with potent effects on intestinal epithelial cells, as well as constituents of the mucosal immune system. For example, TGF-β has been demonstrated to effect marked inhibition of intestinal epithelial cell proliferation and possibly differentiation, as well as striking effects on functional features of B and T lymphocytes and monocyte populations. These observations lend support to the emerging impression of the important integration of the epithelial surface of the mucosa with the mucosal immune system.

The extent of the integration of the epithelial cell populations of the intestine and the constituents of the lamina propria has not been fully defined. Most notably, a number of powerful cytokines have been presumed to modulate a circumscribed spectrum of targets without delineation of their effects on intestinal epithelial cells. In this report, we demonstrate that intestinal epithelial cells express functional specific receptors for IL-2, a potent cytokine whose biological targets have been presumed to be largely limited to lymphocyte and macrophage populations (11, 12). These findings suggest that this cytokine can facilitate coordinated response by epithelial cells and more classical cellular constituents of the mucosal immune system present within the lamina propria.

Methods

Cell culture and IL-2 treatment. IEC-6 cells (13) 14–17th passage and IEC-17 cells (American Type Culture Collection, Rockville, MD) were routinely maintained in DME containing 5% fetal calf serum. In experiments assessing the effects of IL-2 on cells, subconfluent monolayers were kept in DME containing 0.1% fetal calf serum for 17 h before

addition of 10–100 U/ml recombinant human IL-2 (Collaborative Biomedical Products, Bedford, MA) and culture continued for 6–48 h.

IL-2 binding by IEC-6 cells. Subconfluent IEC-6 cells were trypsinized, harvested, and seeded in 24-well plates (5×10^4/well). After 10 h, the cells were resuspended in DME with 0.1% fetal calf serum. Medium was changed after 20 h and 1% albumin and human recombinant ^{125}I-IL-2 (specific activity 33.7 μCi/μg; Du Pont Pharmaceuticals, Wilmington, DE) alone or together with a 100-fold excess of nonlabeled IL-2 was added to the cells maintained at 37° at different time points. In additional controls, cells were coincubated with either immunoneutralizing antibody (0.1 ml; 15.3 mg/ml) to rat IL-2 receptor (OX-39; Serotec Ltd., Kiddington, United Kingdom) or an isotype-matched control monoclonal antibody. Subsequently the cells were washed three times with PBS, followed by addition of 10% TCA before assessment of radiolabeled ligand in the resulting precipitate. Scatchard plot analysis was carried out after similar assessment of binding using a mixture of ^{125}I-IL-2 and nonlabeled IL-2 in concentrations varying from 0.005 to 10 nM. After 30 min, the cells were washed with PBS, TCA precipitated, and the labeled ligand in the precipitate determined in a gamma counter.

Northern blot analysis of TGF-β and mac-2 mRNA expression. IEC-6 cells (treated or untreated with IL-2) were harvested and RNA extracted by modification of the method of Chirgwin et al. (14) in which extraction buffer was added and samples were immediately frozen on dry ice before further processing. Generally, RNA was extracted from 6 × 60-mm plates for each determination. mRNA (3.5 μg/lane) obtained by oligo-dT affinity chromatography was electrophoresed in a 1% agarose gel. After blotting onto nitrocellulose paper (MSI, Westboro, MA), hybridization was carried out overnight at 42°C in the presence of 50% formamide, 5× SSC, and 10% dextran sulfate. Rat TGF-β cDNA was labeled as previously described (7, 15, 16). A probe, mac 2.16, specific for the lectin mac-2 expressed by intestinal epithelial cells (as well as macrophages) was obtained as a gift from Dr. Shiv Pillai and labeled by the same method (17). The blots were first hybridized with either the TGF-β cDNA or mac-2 cDNA probe, washed (55°, 2× SSC, 0.1% SDS), and exposed overnight at −80°C. The same blots were then hybridized with the GAPDH probe to standardize mRNA loading as previously described (7, 18). Hybridization was quantitated by scanning densitometer.

Western blot analysis of IL-2 receptor protein. IEC-6 cells were harvested using a rubber policeman. Primary intestinal epithelial cells were obtained by the method of Weiser (19). Rat splenocytes were obtained, incubated for 24 h in the presence of PHA and then used as a positive control. IEC-6 and primary cells were washed twice with PBS and collected in SDS-polyacrylamide gel sample buffer. The cells were sonicated, centrifuged and the supernatant loaded onto a 10% SDS polyacrylamide gel and electrophoresed essentially by the method of Laemmli (20). The gel was blotted onto nitrocellulose filters and stained for IL-2 receptor protein by avidin-biotin-peroxidase staining procedures using an anti-IL-2 receptor protein antisera (Amersham Corp., Arlington Heights, IL) and commercially available staining reagents (Vectastain ABC; Vector Laboratories, Inc., Burlingame, CA).

Northern blot analysis of IL-2 receptor mRNA. Probes specific for the rat IL-2 receptor β and α chains were prepared from mononuclear cells obtained from rat spleen by PCR: after reverse transcription of mRNA from PHA-stimulated rat spleen mononuclear cells using avian myeloblastosis virus reverse transcriptase, PCR was carried out with primers (5′:AAC GAC TGT TCC CAT CTT AA and 3′:AAA TTC CAA GTA TGG ATT AA for IL-2 receptor-β [IL-2Rβ])[1] and [5′:CTG CAG AGA ATT TCA TCC A and 3′:AAG GTT CTC CTG GTA CAC AGA for IL-2Rα] based on published sequence of the rat IL-2Rβ and IL-2Rα (21) for 35 cycles (95°C for 1 min, 48°C for 1.5 min, and 72°C for 2 min) followed by 72°C for 8 min to yield inserts of the predicted size (420 and 402 nucleotides) which were then ligated into the TA cloning vector (Stratagene Inc., La Jolla, CA) using methods suggested by the manufacturer. Sequencing of the ligated inserts by the dideoxynucleotide chain termination method confirmed identity of the PCR products with the published putative region of the IL-2Rβ and IL-2Rα chains. Northern blot analysis was carried out using these probes after labeling (16). Primary enterocytes (1° IEC) were isolated by the method of Weiser (19). Villus (fractions no. 1 and 2) and crypt (fractions no. 8 and 9) epithelium were depleted of contaminating intraepithelial lymphocytes by sequential fractionation on Percoll gradients. Cells were first suspended in 25% Percoll in RPMI media, centrifuged (600 *g*, 20 min) and epithelial cells recovered at the top were washed, resuspended in 44% Percoll in RPMI, layered over a 67.5% Percoll gradient, and again centrifuged to recover purified epithelial cell populations. Absence of lymphocytes was confirmed by lack of staining antibody in cytospin preparations of the epithelial cell populations with the MRC OX8 antibody; (positive staining of rat intraepithelial and lamina propria CD8-positive lymphocytes was confirmed by staining of normal rat small intestine). Northern blot analysis was carried out using the IL-2R probes and mRNA (2 μg/lane) obtained from these primary epithelial cells, as well as the IEC-6 cell line and PHA-stimulated rat splenic lymphocytes as a positive control in conditions detailed for analysis of TGF-β mRNA.

Determination of thymidine incorporation in IEC-6 cells. Subconfluent IEC-6 cells maintained in 35 mm plastic dishes were changed to fresh DME containing 0.1% fetal calf serum 17 h before addition of IL-2 (10 or 100 U/ml). A mouse anti-rat IL-2 receptor monoclonal antibody designated OX-39 (10 μl) was added alone or in conjunction with IL-2 (100 U/ml). ^{3}[H]thymidine (6 μCi/dish) was added at varying times after addition of IL-2 and incorporation determined after further incubation for 4 h by standard techniques (8). Results were defined as cpm/10^5 cells (mean±SEM); statistical analysis was performed using the Student's *t* test for paired data.

Results

The IEC-6 cell line has served as a useful tool to study intestinal epithelial cell biology. Extensive studies over the past several years have demonstrated the similarity between this cell line and the normal rat crypt intestinal epithelial cell (13). Although important distinctions between this cell line and its primary cell counterpart must be assumed in view of the limited capacity to undergo terminal differentiation into a phenotypically mature villus enterocyte, nonetheless this nontransformed epithelial cell line offers the opportunity to explore cell responses without the confounding presence of other cellular constituents which could contaminate preparations of primary intestinal epithelial cells.

The presence of IL-2 receptor was first evaluated through assessment of specific binding of ^{125}I-IL-2 to IEC-6 cells. As illustrated in Fig. 1, IEC-6 cells possess specific binding sites for the ^{125}I-IL-2 ligand; binding of IL-2 was maximal within 30 min. The specificity was confirmed by the ability to inhibit binding by coincubation of the radioligand with either excess cold ligand or an antibody directed against the rat IL-2 receptor. Scatchard plot analysis suggests that cells previously unexposed to IL-2 exhibit two distinct populations of receptors of intermediate and relatively low affinity with apparent approximate K_d of 10^{-9} and 10^{-8} M and a calculated 1.3×10^4 and 1×10^5 receptors per cell, respectively.

Specific IL-2 binding sites appear to correspond to previously described IL-2 receptor protein by Western blot analysis. As demonstrated in Fig. 2, Western blot analysis using an antibody specifically recognizing the IL-2 receptor results in spe-

1. *Abbreviation used in this paper:* IL-2R, IL-2 receptor.

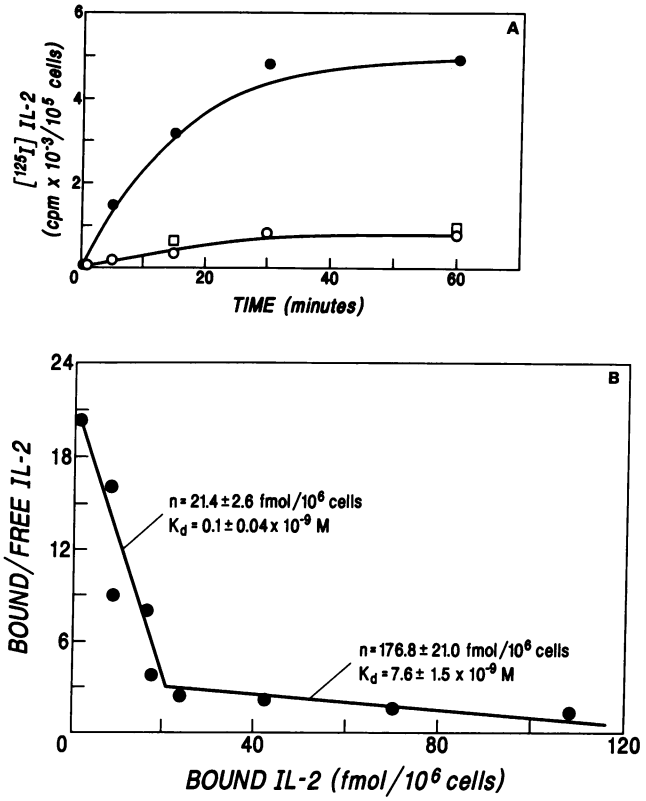

Figure 1. (*A*) Specific binding of IL-2 by a rat intestinal epithelial cell line. Subconfluent IEC-6 cells were trypsinized, harvested, and seeded in 24-well plates (5×10^4). After 10 h, the cell media was changed to DME with 0.1% fetal calf serum. Medium was changed after 20 h and human recombinant ^{125}I-IL-2 was added to the cells alone (●) or together with either a 100-fold excess of nonlabeled IL-2 (○) or mouse monoclonal antibody to the rat IL-2 receptor, 0.1 mg/ml (□) at different time points. Subsequently the cells were washed three times with PBS, followed by addition of 10% TCA before assessment of radiolabeled ligand in the resulting precipitate. (*B*) Scatchard plot analysis of ^{125}I-IL-2 binding to IEC-6 cells. A mixture of ^{125}I-IL-2 and nonlabeled IL-2 in concentrations varying from 0.005 to 10 nM was added to the cells prepared as described in legend to (*A*). After 30 min the cells were washed with PBS, TCA precipitated, and the labeled ligand in the precipitate was counted in a gamma counter.

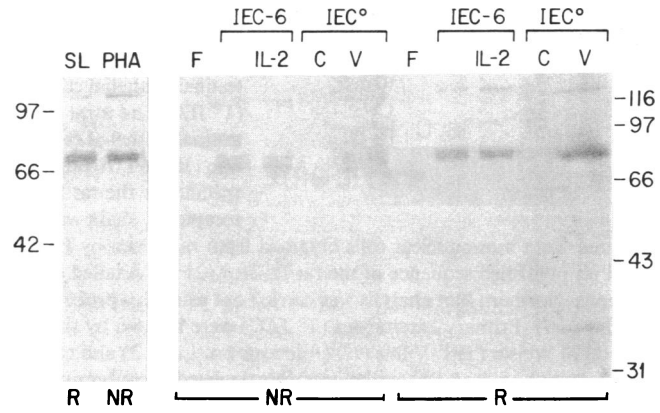

Figure 2. Western blot of IL-2 receptor proteins in IEC-6 cells and rat primary intestinal epithelial cells. Western blot analysis of subconfluent IEC-6 cells, untreated and after 24 h of treatment with 20 U/ml of human recombinant IL-2 (− or +) in reducing and nonreducing conditions. On the same blot, freshly isolated primary rat intestinal cells (*IEC°*), the IEC-6 cell line and as control (*F*) a rodent fibroblast line (*BHK*) in reducing (*R*) and non-reducing (*NR*) conditions. PHA-stimulated lymphocytes from rat spleen are also indicated (*SL + PHA*).

Primary intestinal cells were obtained by the method of Weiser (19); lymphoid cells (10^7) were isolated from the rat spleen and stimulated with PHA for 24 h. IEC-6 and primary cells were washed twice with PBS, and collected in SDS-polyacrylamide gel sample buffer. The cells were sonicated, centrifuged and the supernatant loaded into a 10% SDS polyacrylamide gel and electrophoresed essentially by the method of Laemmli (20). The gel was blotted onto nitrocellulose filter and stained for IL-2 receptor by avidin-biotin-peroxidase staining procedure.

cific staining of a 75-kD species among proteins solubilized from IEC-6 cells. No binding was observed to proteins solubilized from a rodent fibroblast line used as negative control. Thus, the Western blot analysis reveals one β chain receptor protein thought to contribute to low and intermediate affinity binding sites on lymphocytes (11, 12). In contrast to T lymphocytes in which IL-2 leads to enhanced expression of IL-2 receptors, only a minimal increase in apparent abundance of the IL-2 receptor was noted after pretreatment with IL-2. Most importantly, the IL-2 receptor protein was also found in primary rat enterocytes isolated by the method of Weiser (19). The apparent abundance of the 75-kD IL-2 receptor subunit in primary cells assessed by Western blot varied as a gradient, with highest concentrations in the differentiated villus cell population and substantially less receptor protein in the undifferentiated mitotically active crypt cells (Fig. 2).

The observed IL-2β receptor chain protein found in primary enterocytes by Western blot analysis was paralleled by the expression of mRNA for rat IL-2 receptor β chain. mRNA was prepared from primary enterocytes after thorough depletion of initial isolated cell preparations of contaminating intraepithelial lymphocytes by two cycles of Percoll gradient centrifugation. Depletion of detectable intraepithelial lymphocytes was confirmed by the absence of immunocytochemical staining of cytospin preparations with a monoclonal antibody (0X 8) which recognizes intraepithelial lymphocytes. As demonstrated in Fig. 3, Northern blot analysis of mRNA from primary rat enterocytes as well as IEC-6 cells demonstrated the presence of a specific 3.0-Kb transcript. In contrast, no mRNA specific for the rat IL-2 receptor α chain (data not shown) could be detected by this approach.

The presence of IL-2 specific receptors on IEC-6 cells suggests that the growth and/or functional state of these cells may actually be modulated by this factor previously thought to be largely directed toward lymphocyte and monocyte populations. In the context of efforts to understand the range of protein factors which may regulate growth and differentiation of intestinal epithelial populations, the effect of IL-2 on the expression of TGF-β_1 in IEC-6 cells was examined. Studies in this laboratory and elsewhere have suggested that TGF-β_1 is an important growth regulating factor in intestinal epithelial cell populations (7–10). TGF-β_1 has been found to be expressed by primary enterocytes and to effect strong inhibition of proliferation of the IEC-6 cell line. Recent studies in this laboratory have provided evidence of interrelated autocrine and paracrine expression of TGF-β_1 and TGF α in these cells (7). As illus-

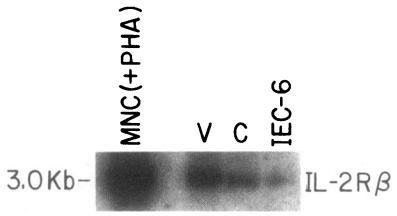

Figure 3. Expression of IL-2 receptor β chain mRNA in primary intestinal epithelial cells (1° IEC) and a rat intestinal epithelial cell line (IEC-6). A probe specific for the rat IL-2 receptor β_1 chain was prepared from mononuclear cells obtained from rat spleen by PCR: based on published sequence of the rat IL-2Rβ (21) as detailed in Methods. Northern blot analysis was carried out using this probe after labeling (16). Primary enterocytes (*1° IEC*) were isolated by the method of Weiser (19). Villus (*V*) (fractions no. 1 and 2) and crypt (*C*) (fractions no. 8 and 9) epithelium were depleted of contaminating intraepithelial lymphocytes by sequential fractionation on Percoll gradients. Northern blot analysis was carried out using the IL-2Rβ probe and mRNA (2 μg/lane) obtained from these primary epithelial cells as well as the IEC-6 cell line and PHA stimulated rat splenic lymphocyte (*MNC + PHA*) as a positive control.

trated in Fig. 4, IL-2 led to significant enhancement of TGF-β_1 expression in the IEC-6 cell line. IL-2 appeared to be especially potent in inducing expression of the TGF-β_1 transcript with enhancement ranging from 2–50-fold in eight separate experiments when mRNA levels were standardized relative to the constitutive marker transcript for glyceraldehyde-3-phosphate dehydrogenase. Enhanced expression was most notable after IL-2 treatment of serum starved (0.1% serum) cells but could also be detected in cells maintained in standard (5%) fetal calf serum concentrations. The effect of IL-2 was dose dependent, with optimal response found at concentrations of 100 U/ml or greater. Increased expression of TGF-β_1 was apparent within 6 h after addition of IL-2 and persisted as long as 48 h. To confirm functional response to IL-2, the effect of the ligand on expression of another gene transcript was assessed. Modification of mRNA expression by IL-2 was not unique to TGF-β, IL-2 also enhanced content of mRNA encoding the galactoside binding protein mac-2 in IEC-6 cells. Importantly, another intestinal epithelial cell line designated IEC-17 appeared similarly responsive to IL-2; e.g., induction of mac-2 (Fig. 5). These findings indicate that the response to IL-2 is not an idiosyncratic characteristic of the IEC-6 cell line. These observations directly indicate that IL-2 receptors on IEC-6 cells are indeed functional.

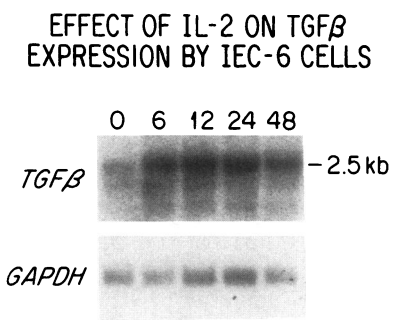

Figure 4. Induction of TGF-β_1 expression in IEC-6 cells by IL-2. Northern blot analysis of mRNA from IEC-6 treated with IL-2 (10 U/ml). Subconfluent cell monolayers kept in 0.1% fetal bovine serum DME for 17 h before the addition of IL-2, were harvested at varying times after addition of IL-2. Northern blots prepared as described in Methods was hybridized sequentially with probes for TGF-β_1 and the constituitive marker GAPDH.

The impact of IL-2 on the IEC-6 intestinal epithelial cell line was further examined through assessment of incorporation of tritiated thymidine to confirm the functional capacity of IL-2 receptors. Previous work in this laboratory has established a close correspondence between thymidine incorporation and cellular proliferation defined by actual cell counts. IL-2 effected stimulation of thymidine incorporation from 6 to 12 h after addition of this factor to serum starved IEC-6 cells. As detailed in Table I, although thymidine incorporation transiently increased (24 h), progressive inhibition was observed between 24 and 48 h after addition of IL-2, perhaps reflecting accumulation of bioactive TGF-β_1. The initial stimulation and subsequent inhibition were more pronounced at higher concentrations of IL-2. Both effects could be abrogated by addition of a neutralizing anti-rat IL-2 receptor antibody at the time of addition of IL-2. Cells remained subconfluent throughout the interval of IL-2 treatment, indicating that decreased thymidine incorporation was not related to achieving confluence in culture.

Discussion

The gastrointestinal mucosal immune response system has been recognized to encompass a diverse mixture of cells within the lamina propria whose functional properties are both mediated and modulated by a highly complex range of cytokine proteins. In the past few years, a number of observations have supported the concept that the epithelial cell population, which comprises the mucosal surface, interacts with the classical elements of the mucosal immune system through the cytokine network. These observations include the demonstration that intestinal epithelial cells possess functional receptors for some cytokines (e.g., IL-1 and IFN-α) and themselves produce (as well as respond) to other factors which may also affect lamina propria cell populations; e.g., TGF-β_1 and interleukin-6 (2–10). In addition, intestinal and colonic epithelial cells in man and animal have been found to express MHC class II antigens as well as Cd1, either on a constituitive basis or in association with inflammation suggesting that they can serve as antigen-presenting cells.

In this report, we demonstrate that presumptively normal rat intestinal epithelial cells possess functional receptors for the

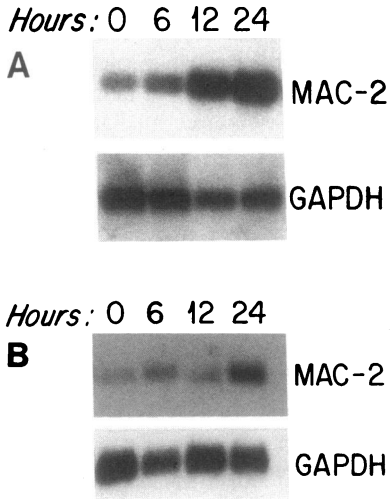

Figure 5. Induction of mac-2 expression by IL-2. Northern blot analysis of mRNA from either IEC-6 (*A*) or IEC-17 (*B*) treated with IL-2, 10 U/ml. Subconfluent monolayers kept in 0.1% fetal calf serum containing DME for 24 h before the addition of IL-2, were harvested at varying times after addition of IL-2. Northern blots prepared as described in Methods was hybridized sequentially with probes for mac-2 and the constituitive marker GAPDH.

Table I. Effect of IL-2 on Thymidine Incorporation in IEC-6 Cells

Addition*	Hours	
	24	48
	[^{3}H]thymidine incorporation (cpm±SEM)	
None	18,860±1,270	34,750±2,490
IL-2 (10 U)	24,320±1,100	18,470±2,470
(100 U)	46,470±2,610	9,590±1,480
IL-2 (100 U) + anti-IL-2Rβ Ab‡	16,180±950	27,100±1,930
Anti-IL-2Rβ Ab	18,420±1,120	29,970±2,740

* Semiconfluent IEC-6 cells were maintained in 0.1% fetal calf serum containing DME in 35-mm plastic dishes to which IL-2 alone, IL-2 plus 0.1 mg/ml anti-IL-2 receptor β chain antibody, or equivalent volume buffer were added in quadruplicate in six separate experiments. Thymidine incorporation assessed at 24 and 48 h by addition of ^{3}[H]thymidine (6 μCi/dish), and determination of incorporation of radiolabel into TCA-precipitable material by described techniques (8).

‡ Antibody to IL-2 receptor β chain.

potent cytokine IL-2. In demonstrating the potential response of intestinal epithelial cells to this cytokine, which is central to immune activation, these findings suggest that the intestinal epithelial cell population may be closely integrated with mucosal immune response. Thus, the IEC-6 line, a nontransformed epithelial cell line derived from the rat intestine was found to exhibit specific IL-2 binding in association with the demonstration of both specific IL-2 receptor protein by Western blot and IL-2 receptor mRNA by Northern blot analysis. The relevance of these studies are supported by the finding of the IL-2 receptor in primary isolated rat intestinal epithelium in which the amount of receptor was present in a gradient with highest levels associated with the villus cell populations. The latter implies that the IL-2 receptor was unlikely to reflect contamination with intraepithelial lymphocytes, which are randomly distributed in the epithelium and lack IL-2 receptors themselves. It should be noted that epithelial populations obtained by the method of Weiser (19) have been found to be devoid of lamina propria contamination (22). In addition, initial cell populations were specifically depleted of any contaminating intraepithelial lymphocytes by sequential Percoll gradient centrifugation prior to Northern blot analysis. Finally, the detection of the receptor protein and a specific IL-2 receptor β chain transcript in the established IEC-6 cell line in which confounding contamination can be discounted entirely, lends further support to this conclusion.

These studies provide some insight into the composition of IL-2 receptors in these epithelial cells. As noted, both Western and Northern blot analysis confirm the presence of the IL-2Rβ chain. The IL-2Rβ is a 75-kD protein with an intracellular domain that is important for signaling and an extracellular domain that binds IL-2 with relatively low affinity (23, 24). The absence of detectable IL-2Rα chains is consistent with binding kinetics, which suggested the absence of high affinity receptors that require the presence of both α and β chains. The presence of both intermediate and low affinity binding is consistent with the presence of the IL-2Rγ chain which can enhance the affinity of receptors comprised of IL-2Rβ dimers by decreasing the ligand off-rate (24, 25). This can also contribute to signal transduction. However, the rat IL-2Rγ has not yet been cloned, precluding direct confirmation of this inference at this time.

Most importantly, the IEC-6 cell line permits an assessment of the functional integrity of IL-2 binding sites, which is not feasible in primary cell populations due to the inability to sustain them in vitro. Indeed, IL-2 was found to effect at least two cellular responses. Increased expression of TGF-β_1 was apparent within 6 h after addition of IL-2 and persisted as long as 48 h. It is possible that the sustained enhancement in TGF-β_1 expression observed 6–48 h after IL-2 addition reflects the synergistic effect of the autocrine induction of TGF-β_1. Recent work in this laboratory has demonstrated TGF-β_1 stimulation of its own expression in this cell line (7). However, the relatively delayed emergence of enhanced TGF-β_1 expression suggests that stimulation may depend on the intermediate induction of as yet unknown genes. While clarification of the early transcriptional responses of IEC-6 cells to IL-2 and their role in controlling TGF-β expression are important features for more detailed studies, these observations in conjunction with a biphasic alteration in thymidine incorporation after addition of the IL-2 ligand indicate that the IL-2 receptors on IEC-6 are functional.

Undoubtedly, IL-2 could lead to additional functional alterations in intestinal epithelial cells as well. These studies were not intended to define the full complement of intestinal epithelial cell responses to IL-2. The effect of IL-2 on mac-2, a galactose binding lectin recently recognized to be expressed by intestinal epithelial cells was assessed in IEC-6 cells to confirm the ability of the IL-2 ligand to affect function after binding (17, 26). It is important to note that a similar response was also observed when another independently derived rat intestinal epithelial cell line was exposed to IL-2. These observations in conjunction with the apparent presence of IL-2–specific receptors on primary cell populations supports the notion that these responses reflect physiologic activities and not an aberrant characteristic of a single cell line. Further studies will be needed to define the dimensions of functional response of intestinal epithelial cells to IL-2 in a comprehensive manner.

The findings noted in this report demonstrate that IL-2 may have a broader spectrum of important cellular targets than previously appreciated. Although IL-2 receptors have been described on corneal epithelium and on some cells within the nervous system (27, 28), its actions have been largely presumed to be focused on lymphocyte and macrophage populations. The present observations identify a large and distinct population of cells that are found in close proximity to major components of the mucosal immune system. Intestinal epithelial response to IL-2 may have important ramifications in vivo for the understanding of the regulation of immune response in normal tissue and a variety of inflammatory diseases such as inflammatory bowel disease. Further work will be needed to characterize the full effects of IL-2 on intestinal epithelial cells and the mechanism of intestinal epithelial cellular response to this interleukin.

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