Human intestinal epithelial cells express functional cytokine receptors sharing the common γc chain of the interleukin 2 receptor

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ABSTRACT Interleukin (IL) 2 signaling requires the dimerization of the IL-2 receptor β (IL-2RB) and common γ (γc) chains. The γc is also a component of the receptors for IL-4, IL-7, and IL-9. To assess the extent and role of the receptor signal transducing system utilizing the γc chain on human intestinal epithelial cells, the expression of γc, IL-2RB, and receptor chains specific for IL-4, IL-7, and IL-9 was assessed by reverse transcription-coupled PCR on human intestinal epithelial cell lines and on isolated primary human intestinal epithelial cells. Caco-2, HT-29, and T-84 cells were found to express transcripts for the γc and IL-4R chains constitutively. IL-2RB chain expression was demonstrated in Caco-2 and HT-29 but not in T-84 cells. None of the cell lines expressed mRNA for the IL-2Ra chain. After stimulation with epidermal growth factor for 24 h Caco-2, HT-29, and T-84 cells expressed transcripts for IL-7R. In addition, Caco-2 and HT-29 cells expressed mRNA for the IL-9R. Receptors for IL-2, IL-4, IL-7, and IL-9 on intestinal epithelial cell lines appeared to be functional; stimulation with these cytokines caused rapid tyrosine phosphorylation of proteins. The relevance of the observations in intestinal epithelial cell lines for intestinal epithelial function in vivo was supported by the demonstration of transcripts for γc, IL-2RB, IL-4R, IL-7R, and IL-9R in primary human intestinal epithelial cells.

Interleukin (IL) 2 appears to play a pivotal role in regulating immune cell populations, exerting its effects through binding to specific cell surface receptors. The IL-2 receptor (IL-2R) consists of three membrane proteins: the IL-2Ra chain, the IL-2RB chain, and the common γ (γc) chain (1). Different combinations of these receptors on human cells give rise to high-, intermediate-, and low-affinity binding sites for IL-2 (1). The IL-2RB and γc chains are required for ligand internalization and signal transduction (2). The γc chain has been shown to dimerize also with the IL-4R (3), IL-7R (4), and IL-9R (5) chains to form signal transducing receptors for these cytokines.

The cytokines utilizing the γc chain in their receptor complexes have been identified (1–5) through their regulatory effects on T and B lymphocytes. Little is known about the function of these cytokine receptors and their intracellular signaling pathways in nonhematopoetic cells. In this report we demonstrate that intestinal epithelial cells are able to utilize the γc receptor signal transducing system. Both human intestinal epithelial cell lines and primary human intestinal epithelial cells are able to express transcripts for γc, IL-2RB, IL-4R, IL-7R, and IL-9R chains. These receptors are functional, as demonstrated by the initiation of tyrosine phosphorylation of proteins within intestinal epithelial cells by IL-2, IL-4, IL-7, and IL-9. Intestinal epithelial cells may be integrated into the mucosal immune system by their ability to respond to cytokines produced by classical immune cells.

MATERIALS AND METHODS

Cell Culture. Caco-2, HT-29, and T-84 cells were obtained from the American Type Culture Collection and used for experiments after they had reached confluence for 1 week. Caco-2 and HT-29 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 4 mM L-glutamine (Sigma) in tissue culture plates (Costar). T-84 cells were cultured in DMEM/F-12 medium (GIBCO) containing 10% fetal calf serum. For the detection of cytokine transcripts, cells were stimulated by replacement with fresh medium with or without recombinant epidermal growth factor (EGF) (R & D Systems; 100 ng/ml).

Isolation of Primary Intestinal Epithelial Cells. Intestinal epithelial cells were isolated from colonic biopsies by modifications of a technique described by Harrison and Webster (6). Separated epithelial cells were resuspended in DMEM and 50 µl of cell suspension was placed on a caved glass slide and overlayed with mineral oil. Single epithelial cells were then transferred to a second slide with a micropipette (see Fig. 2). RNA from 20 to 40 primary epithelial cells was isolated after addition of 50 µg of carrier rRNA from Escherichia coli W (Sigma).

Reverse Transcription. Total cellular RNA was isolated by a modified acid guanidinium isothiocyanate/phenol/chloroform extraction (7). Total sample RNA was reverse-transcribed by using 200 units of Moloney murine leukemia virus reverse transcriptase (GIBCO), 20 units of RNasin (Promega), 1 µM dGTP, 1 µM dATP, 1 µM dTTP, and 1 µM dCTP, and 1 µg of hexanucleotide random primer (Boehringer Mannheim) in 50 mM Tris-HCl, pH 8.3/75 mM KCl/3 mM MgCl2/10 mM dithiothreitol for 1 h at 37°C.

PCR Amplification. Primers for amplification of transcripts encoding the IL receptor subunits were designed by using Oligo 4.0 software and synthesized on a PCR-Mate DNA synthesizer (Applied Biosystems) (Table 1). The PCR mixture contained 1X PCR buffer (Perkin–Elmer/Cetus, with 1.5 mM Mg), all four dNTPs (each at 50 µM), each 5’ and 3’ primer at 1 µM, and 1 unit of Taq polymerase (Perkin–Elmer/Cetus), in a total volume of 100 µl. The PCR specific for IL-7R transcripts was carried out with the addition of 10 µCi [α-32P]dATP (Amersham; 1 Ci = 37 GBq) per reaction. IL-9R transcripts were detected by using a nested PCR, which was carried out for 20 cycles with the first set of primers, diluted 1:100, and continued with the second set of primers for an additional 30 cycles. Amplification was carried out on a thermal cycler (Perkin–Elmer Wizard). The amplification was confirmed by using ethidium bromide staining of agarose gels.

Abbreviations: IL, interleukin; IL-nR, IL-n receptor; RT-PCR, reverse transcription-coupled PCR; PTK, protein tyrosine kinase; γc chain, common γ chain; EGF, epidermal growth factor; BSA, bovine serum albumin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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Table 1. PCR primers for the detection of cytokine receptor transcripts and control transcripts used in this study

<table>
<thead>
<tr>
<th>PCR product</th>
<th>GenBank accession no. (ref.)</th>
<th>Position of 5' residue*</th>
<th>Sequence (5' → 3')</th>
<th>Product size, bp</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2Rα</td>
<td>K03122 (8)</td>
<td>67</td>
<td>TCTTCCATCCCCACATCC</td>
<td>285</td>
<td>94°C, 60 s/67°C, 50 s/70°C, 60 s</td>
</tr>
<tr>
<td>IL-2Rβ</td>
<td>M26062 (9)</td>
<td>332</td>
<td>TCTGCGGAAAAACCTGC</td>
<td>238</td>
<td>94°C, 60 s/68°C, 50 s/72°C, 60 s</td>
</tr>
<tr>
<td>γc</td>
<td>D11086 (10)</td>
<td>1108</td>
<td>CTTGTTCCCTTCCACGTTT</td>
<td>275</td>
<td>94°C, 60 s/68°C, 50 s/72°C, 60 s</td>
</tr>
<tr>
<td>IL-4R</td>
<td>X52425 (11)</td>
<td>419</td>
<td>ACGGGAACCCAGGAGGAGGG</td>
<td>335</td>
<td>94°C, 60 s/63°C, 50 s/72°C, 60 s</td>
</tr>
<tr>
<td>IL-7R</td>
<td>M29696 (12)</td>
<td>674</td>
<td>AGCGGTCTCGAGAACGAAAC</td>
<td>418</td>
<td>94°C, 60 s/67°C, 60 s/72°C, 60 s</td>
</tr>
<tr>
<td>IL-9R</td>
<td>M84747 (13)</td>
<td>913</td>
<td>CAAATGCTGATGGTTGTA</td>
<td>490</td>
<td>94°C, 60 s/63°C, 60 s/72°C, 60 s</td>
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<tr>
<td>CD45</td>
<td>Y00638 (14)</td>
<td>1856</td>
<td>AGCCCTGTCTTTGTTC</td>
<td>205</td>
<td>94°C, 60 s/58°C, 60 s/72°C, 60 s</td>
</tr>
<tr>
<td>GAPDH</td>
<td>M33197 (15)</td>
<td>419</td>
<td>TCATCTCCTGCCCCCTCT</td>
<td>338</td>
<td>94°C, 60 s/58°C, 60 s/72°C, 60 s</td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
*Numbers beside the primer sequences indicate the most 5'-end nucleotide of the primer corresponding to the sequence for which the GenBank accession number is given.

Perkin-Elmer thermal reactor with 30 cycles for the PCRs utilizing reverse-transcribed RNA from intestinal epithelial cell lines or controls and with 35 cycles for the PCRs with RNA obtained from primary intestinal epithelial cells. PCR products from reverse-transcribed RNA from intestinal epithelial cell lines, obtained with primers for human IL-2Rα, IL-2Rβ, γc, IL-4R, IL-7R, and IL-9R, were cloned into pBluescript SK+ vector (Stratagene) and sequenced by using the Sequenase 2.0 kit (United States Biochemical). Sequence analysis was performed by using MACVECTOR (IBI) and the GCG package of programs. In addition, PCRs were carried out with RNA alone to rule out amplification products derived from genomic DNA.

Immunoblot Analysis. For determination of tyrosine phosphorylation in response to cytokines, HT-29 or Caco-2 cells were grown in 6-well plates (Costar) until they reached confluence for 1 week. Cells were then starved overnight in medium containing 0.1% fetal calf serum and stimulated with IL-2 (100 units/ml) or IL-4 (100 ng/ml), IL-7 (100 ng/ml), or IL-9 (100 ng/ml) (all R & D Systems) as indicated.

After removal of medium, cells were lysed in 1 ml of 0.0625 M Tris-HCl, pH 6.8/2% (wt/vol) SDS/3% (vol/vol) 2-mercaptoethanol/10% (vol/vol) glycerol/100 mM sodium fluoride (Sigma)/2 mM sodium orthovanadate/aprotinin (10 μg/ml)/leupeptin (10 μg/ml)/pepsatin (10 μg/ml)/1 mM phenylmethylsulfonyl fluoride. Cell lysate (50 μl) was separated on a SDS/7.5% polyacrylamide gel and proteins were transferred onto Immobilon-P membranes (Millipore). Equal loading and transfer of proteins was determined by Ponceau S staining. Phosphotyrosine-containing proteins were stained, after blocking with TBST (50 mM Tris-HCl, pH 7.6/150 mM NaCl/0.01% Tween 20)/1% bovine serum albumin (BSA) fraction V (Sigma) for 1 h, with the mouse anti-phosphotyrosine monoclonal antibody PY-20 (Transduction Laboratories, Lexington, KY) diluted 1:500 in TBST/1% BSA for 1 h followed by washing for 30 min with TBST/0.1% BSA and subsequently incubation with horseradish peroxidase-conjugated goat anti-mouse IgG (heavy and light chains) monoclonal antibody (Amersham) diluted 1:5000 in TBST/0.1% BSA/5% (wt/vol) dry milk for 1 h. Antibody reactions were detected with a chemiluminescence detection kit (DuPont/ NEN).

RESULTS

Human Intestinal Epithelial Cell Lines Are Able to Express Transcripts for the γc Chain and the IL-2Rβ Chain. A reverse transcription-coupled PCR (RT-PCR) approach was used to determine whether human intestinal epithelial cells are able to express transcripts encoding receptors for IL-2. Integrity of the RNA for RT-PCR confirmed by amplification of transcripts for constitutively expressed human GAPDH (Fig. 1A). As demonstrated in Fig. 1 depicting the results of RT-PCRs using reverse-transcribed total cellular RNA isolated from the human intestinal epithelial cell lines Caco-2, HT-29, and T-84, all three cell lines constitutively expressed transcripts for the γc chain (Fig. 1A). Caco-2 and HT-29 cells also expressed transcripts for the IL-2Rβ chain, although this transcript could not be detected in T-84 cells (Fig. 1B). The expression of IL-2Rβ was confirmed by using a GAPDH control.
appeared to be upregulated if cells were pretreated with EGF. In contrast, none of the intestinal epithelial cell lines expressed IL-2Rα mRNA (Fig. 1C).

Human Intestinal Epithelial Cell Lines Express Transcripts for the Receptor Chains That Determine Ligand Specificity for IL-4, IL-7, and IL-9. After the observation that T-84 cells expressed mRNA for the γc chain in the absence of detectable transcripts for the IL-2Rβ chain, we hypothesized that intestinal epithelial cells might express other cytokine receptor complexes known to utilize this subunit. Therefore, the same reversed-transcribed RNA was subjected to a PCR with primers for the cytokine binding subunits of the receptors for human IL-4, IL-7, and IL-9. As demonstrated in Fig. 1, mRNA encoding the IL-4R was present in Caco-2, HT-29, and T-84 cells and seemed to be upregulated by EGF (Fig. 1D). RNA encoding IL-7R could only be detected in Caco-2 cells, but after stimulation of cells by EGF, all tumor cell lines expressed mRNA for the IL-7R (Fig. 1E). Similarly, EGF stimulation led to detectable expression of transcript for the IL-9R in two of the cell lines, Caco-2 and HT-29, although they remained undetectable in the T-84 cell line (Fig. 1F). To exclude any environmental contamination with leukocyte-derived RNA, the same reverse-transcribed RNA from intestinal epithelial cell lines was subjected to a PCR for the constant region of the leukocyte common antigen (CD45). As demonstrated in Fig. 1G, none of the mRNA obtained from the three cell lines showed evidence of contamination. In other controls, amplification with RNA alone without prior reverse transcription failed to result in any PCR products, excluding the possibility that the described products represent “artificial” amplifications. PCR products from each cell line were confirmed by subcloning and nucleotide sequencing.

Isolation of Primary Human Intestinal Epithelial Cells. After detection of the cytokine receptors in human intestinal epithelial cell lines, a method was developed to isolate pure intestinal epithelial cells from fresh colonic mucosal biopsies to determine whether the primary epithelial cells express these receptors. Mucosal biopsies were first treated with EDTA to separate whole crypts from the underlying lamina propria (Fig. 2A). Subsequently, the crypts were dissociated into single epithelial cells by incubation with sodium tetraphenylborate (Fig. 2B). Twenty to 40 single epithelial cells were then picked cell by cell with a micropipette, avoiding contaminating cells of nonepithelial origin, and transferred into fresh medium (Fig. 2C). In this way, the intestinal epithelial cells were separated from contaminating cells of nonepithelial origin.

As shown in Fig. 3, intestinal epithelial cells appeared to be free of contamination with lymphocytes and monocytes, as reflected by the inability to detect transcripts for CD45, which was apparent in RNA from peripheral blood leukocyte controls (Fig. 3G). In addition isolated epithelial cells contained no detectable transcripts for the monocyte-lineage-specific marker CD68 or the two constant regions of the T-cell receptor β chain as determined by PCR (data not shown). The sensitivity of this method to detect even minimal contamination was confirmed by the detection of transcripts for CD45 in a preparation of isolated epithelial cells (Fig. 3G, lane 3*), which was intentionally contaminated with two lymphocytes.

Human Intestinal Epithelial Cells Isolated from Colon Biopsies Express Transcripts for IL-2R, IL-4R, IL-7R, and IL-9R Chains. After confirmation of the purity of isolated primary intestinal epithelial cells, these preparations were evaluated for the presence of human γc, IL-2Rβ, IL-2Rα, IL-4R, IL-7R, and IL-9R chains. RT–PCR was carried out with RNA from two primary intestinal epithelial cell isolations (Fig. 3). As controls, 0.1-μg samples of reverse-transcribed total RNA from isolated lamina propria cells, peripheral blood mononuclear cells, or Jurkat cells (for the IL-9R and IL-7R PCR) were used.

As shown in Fig. 3, two representative RNA isolations from primary intestinal epithelial cells were free of contamination with leukocytes RNA (Fig. 3G). These two primary intestinal epithelial cell isolations contained transcripts for the human γc (Fig. 3A and B), IL-2Rβ (Fig. 3B and C), IL-4R (Fig. 3D), and IL-7R (Fig. 3E) chains. In addition, both of the intestinal epithelial cell isolations (isolation 2) also contained transcripts for the IL-9R chain (Fig. 3F). None of the intestinal epithelial cell isolations expressed transcripts for the IL-2Rα chain (Fig. 2C).

Cytokine Receptors Sharing γc Chains Are Functional on Intestinal Epithelial Cells. The signal transduction pathways of cytokine receptors on lymphocytes involves rapid phosphorylation of different cellular substrates. To determine whether cytokine receptors are functional on human intestinal epithelial cells, phosphotyrosine kinase activity in response to IL-2, IL-4, IL-7, and IL-9 was assessed within intestinal epithelial cells.

FIG. 2. Isolation of primary intestinal epithelial cells from colon biopsies. Whole intestinal epithelial cell crypts were removed from underlying lamina propria (A) by incubation with EDTA. Crypts were further processed into single intestinal epithelial cells (B), and finally, single intestinal cells were separated from nonepithelial cells with a micropipette (C).
Western blot analysis with anti-phosphotyrosine antibodies demonstrated a rapid activation of phosphotyrosine kinases in confluent HT-29 cells after stimulation with IL-2 (Fig. 4), IL-4 (Fig. 5), IL-7 (Fig. 6), and IL-9 (Fig. 7). In each instance, after addition of the cytokine ligand, an increase into three overall intensity of phosphorylation of several proteins was apparent. Tyrosine phosphorylation in intestinal epithelial cells in response to cytokines occurred within 2–5 min after stimulation and persisted for up to 1 h.

Tyrosine-phosphorylated proteins in intestinal epithelial cells in response to cytokines could be separated into three groups of 50–70 kDa, 90–120 kDa, and 140–170 kDa. Three proteins between 50 and 70 kDa seemed to be phosphorylated similarly in response to IL-2 (Fig. 4), IL-4 (Fig. 5), IL-7 (Fig. 6), and IL-9 (Fig. 7). Also proteins between 140 and 170 kDa were increasingly phosphorylated after stimulation with either cytokine (Figs. 4–7). In contrast, the proteins of 90–120 kDa were strongly phosphorylated in response to IL-2 (Fig. 4) and IL-4 (Fig. 5) but relatively weakly phosphorylated after stimulation with IL-7 (Fig. 6) and IL-9 (Fig. 7).

DISCUSSION

It has become apparent that intestinal epithelial cells can respond to several cytokines initially recognized to be important in lymphocyte development. Previous studies demonstrated that IL-2 is able to modulate proliferation and enhance the expression of transforming growth factor β by rat intestinal epithelial cell line IEC-6. Furthermore, isolated primary rat epithelial cells expressed IL-2Rb, with highest expression in the differentiated villus cell population (16).

The present studies demonstrate that human intestinal epithelial cells are also able to express receptors for IL-2. Differentiated Caco-2, HT-29, and T-84 cells expressed transcripts for the γc chain and Caco-2 and HT-29 cells also expressed transcripts for the IL-2Rb chain. None of the human intestinal epithelial cell lines expressed transcripts for the IL-2Ra chain. IL-2Rb and γc chains are constitutively expressed in T cells and IL-2Rb is upregulated 5- to 10-fold after T-cell activation. The high-affinity receptor for IL-2 on activated lymphocytes consists of all three receptor chains (1), but
only the IL-2Rβ and γc chains are necessary for the signal transduction of the receptor (2), and intermediate-affinity IL-2Rβ-γc receptors have been described to be functional on natural killer cells (17). Therefore, the expression of IL-2Rβ and γc chains on intestinal epithelial cells is sufficient to enable intestinal epithelial cells to respond to IL-2.

The lack of IL-2Rβ chain expression in the presence of the γc chain in T-84 cells led to the hypothesis that the γc chain on intestinal epithelial cells is shared in receptor complexes for other cytokines, as described for lymphocytes (3-5). Caco-2, HT-29, and T-84 cells expressed high levels of transcripts for the IL-4R chain. IL-4 was previously reported to upregulate the expression of polymeric immunoglobulin receptor on epithelial cells synergistically with interferon γ (18). IL-4 and interferon γ are also able to decrease the epithelial barrier function of T-84 cell monolayers (19).

In response to stimulation with EGF, intestinal epithelial cells were also able to express transcripts for the IL-7R and IL-9R chains. Previously, IL-7 (8) and IL-9 (9) have been reported to modulate T-cell and B-cell proliferation and differentiation; but expression of their receptors on epithelial cells was not previously observed.

The physiological relevance of the receptors detected in human intestinal epithelial tumor derived cell lines is suggested by the presence of transcripts in isolated intestinal epithelial cells from colon biopsies. The latter found to be free of mRNA for the leukocyte common antigen (CD45) excluding possible contamination with any cells belonging to the leukocyte lineage. Collectively, these observations suggest that secretion of IL-2, IL-4, IL-7, and potentially IL-9 during mucosal immune response and intestinal inflammation not only would affect lamina propria macrophages and lymphocytes but also may influence epithelial cell function.

The present findings demonstrate that the receptors for IL-2, IL-4, IL-7, and IL-9 on intestinal epithelial cells are activated by their cognate ligands. Although lacking intrinsic kinase activity, these receptors couple ligand binding to induction of tyrosine phosphorylation of cellular substrates. The phosphorylation of γc (10), IL-2Rβ (11), and IL-4R (12) chains has been observed. Two major families of nonreceptor tyrosine kinases have been linked to cytokine receptor complexes utilizing the common γc chain. The first family includes the src family of protein tyrosine kinases (PTKs) and the second includes the Jak PTK family.

The proteins phosphorylated in response to IL-2, IL-4, IL-7, and IL-9 in human intestinal epithelial cell lines of 50–70 kDa may belong to the src PTK family. The IL-2R has been shown to associate with p56Lck (13), p53/p56lyn (14), and p59Fyn (15), and the IL-4R has been shown to associate with p53/p56lyn and p59Fyn (20). Phosphorylated proteins of 90–120 kDa in human intestinal epithelial cell lines may encompass Jak PTKs. Jak1 and Jak3 have recently been shown to associate with IL-2Rβ and γc chains (5, 21) and IL-2, IL-4, IL-7, and IL-9 induced tyrosine phosphorylation and in vitro kinase activity of Jak1 and Jak3 (5). It should be noted that different proteins of 90–120 kDa were phosphorylated by various cytokines, suggesting different signaling pathways may be used by individual cytokines in human intestinal epithelial cell lines. Phosphorylated proteins between 140 and 170 kDa have been demonstrated in other cell lines in response to IL-2 (6), IL-4 (22), and IL-7 (23). However, none of these proteins, with the exception of the 140-kDa IL-4R chain (12) have been identified.

Further experiments are necessary to determine whether the signaling pathways for cytokine receptor complexes utilizing the common γc chain differ between intestinal epithelial cells and hematopoietic cells. The expression of the cytokine receptor for IL-2, IL-4, IL-7, and IL-9 on intestinal epithelial cells may permit a highly plastic pathway of intestinal epithelial cell–lymphocyte interaction, which may prove critical for the function of the mucosal immune system.

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