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Interleukin 2 (IL-2) and interleukin 7 (IL-7) reciprocally induce IL-7 and IL-2 receptors on γδ T-cell receptor-positive intraepithelial lymphocytes

(cytokine receptor/mRNA/quantitative RT-PCR)

KOHTARO FUJIHASHI*, SHIGETADA KAWABATA*, TAKACHIKA HIROI*†, MASAFUMI YAMAMOTO*, JERRY R. MCGHEE‡, SHIN-ICHI NISHIKAWA§, AND HIROSHI KIYONO*†¶

Departments of *Oral Biology and ‡Microbiology, Immunobiology Vaccine Center, University of Alabama at Birmingham Medical Center, Birmingham, AL 35294; †Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan; and §Department of Molecular Genetics, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

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ABSTRACT **In this study, we describe the interaction between cytokine and cytokine receptor (R) for the activation and proliferation of γδ T-cell receptor-positive T cells (γδ T cells). γδ T cells isolated from murine intestinal intraepithelial lymphocytes (IELs) were separated into $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ fractions according to the intensity of γδ T-cell receptor expression. The $\gamma\delta^{Dim}$ T cells express low levels of IL-2R and IL-7R as shown by flow cytometry and reverse transcriptase–PCR analysis, whereas $\gamma\delta^{Bright}$ T cells did not express either receptor. Our study also revealed that recombinant murine (rm)IL-2 and rmIL-7 reciprocally induced high expressions of IL-7R and IL-2R, respectively, on $\gamma\delta^{Dim}$ T cells but not on $\gamma\delta^{Bright}$ IELs. Thus, treatment of $\gamma\delta^{Dim}$ T cells with rmIL-2 and rmIL-7 resulted in high proliferative responses, whereas $\gamma\delta^{Bright}$ T cells did not respond to these two cytokines. The sources of these two cytokines for $\gamma\delta^{Dim}$ T cells were neighboring epithelial cells (IL-7) and αβ IELs (IL-2 and IL-7). Cytokine signaling by IL-2 and IL-7 from αβ T cells and epithelial cells was necessary for the expression of IL-7R and IL-2R, respectively, on a subset of γδ T cells (e.g., $\gamma\delta^{Dim}$ T cells) in mucosa-associated tissue for subsequent activation and cell division.**

The mucosa-associated tissues including the gastrointestinal tract are continuously and directly exposed to ubiquitous environmental antigens via the epithelium. A large number of $CD3^+$ T cells reside in this intestinal epithelium and are commonly termed intraepithelial lymphocytes (IELs). It has been estimated that one $CD3^+$ T cell can be found for every six epithelial cells (1). Although IELs possess several unique characteristics when compared with $CD3^+$ T cells in other organized systemic lymphoid tissues, the most profound feature of IELs is the occurrence of high numbers of γδ T cells. Thus, it has been shown that 20–80% of IELs expressed γδ heterodimer chains of T-cell receptors (TCRs) dependent on age, strain, and microenvironment (2–6). However, it is now generally agreed that an approximately equal frequency of γδ and αβ T cells are seen in IELs isolated from young adult mice (4–6). Despite the numerous attempts that have been made to understand thymic and extrathymic development of γδ T cells in murine IELs, little information is currently available regarding the biological role of γδ T cells.

To understand the immunological function of γδ T cells in IELs, it was important to examine the interaction between cytokine and cytokine receptor (R) for γδ T cells. An array of cytokines, including IL-1, IL-2, IL-4, and IL-6, have been shown to have roles in regulating T-cell growth, activation, and proliferation (7–10). Among these interleukins, IL-2 was originally isolated as a T-cell growth factor and appeared to stimulate T-cell growth directly without a requirement for other cytokines (8). Recently, it was also shown that IL-7 can act on lymphocytes of the T-cell lineage, despite the fact that this cytokine was initially discovered by its ability to initiate proliferation of B-cell precursors (11–13). Thus, this cytokine was originally considered as a pre-B-cell growth factor (11, 12). However, this 25-kDa cytokine has been reported to enhance anti-CD3 and lectin-induced proliferative responses of mature T cells (14–16). Further, it was also suggested that IL-7 may play an important role in T cell ontogeny because IL-7-specific mRNA has been demonstrated in murine thymus (12). In addition, IL-7 induced proliferative responses in cultures containing fetal thymic T-cell precursors and thymocytes (17–19). It has been shown that IL-2 and IL-7 were thought to serve as complementary T-cell activation factors for thymus-derived γδ T cells in addition to αβ T cells (20). Furthermore, because a combination of IL-2 and IL-7 induce high proliferative responses in peritoneal γδ T cells isolated from *Listeria*-infected mice in the presence of peritoneal macrophages (21), it was important to examine whether γδ T cells isolated from intestinal epithelium respond to these cytokines. To this end, we have found a reciprocal interaction between IL-2 and IL-7 for IL-2R and IL-7R expression in the regulation of γδ IEL responses.

MATERIALS AND METHODS

Isolation of Epithelial Cells, γδ, and αβ IEL T Cells. IELs were isolated by a standard mechanical dissociation method followed by a discontinuous Percoll gradient from C3H/HeN mice (8–10 weeks old) (5, 6, 22, 23). IELs were then stained with fluorescein isothiocyanate-conjugated monoclonal antibody (mAb) anti-γδ TCR (GL3; PharMingen) or mAb anti-αβ TCR (H57–597; PharMingen) and then subjected to flow cytometry (FACStar^{Plus}; Becton Dickinson) for cell analysis and separation. The purity of separated subsets was determined by FACStar^{Plus}; the subsets contained >99% pure γδ and αβ T cells. In some experiments, epithelial cells were isolated from the small intestine. For this purpose, Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (Sigma)/1.5 mM EDTA was used as the dissociation medium (24). Following a

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Abbreviations: IELs, intraepithelial lymphocytes; IL-2, interleukin 2; IL-7, interleukin 7; rm, recombinant murine; R, receptor(s); RT, reverse transcriptase; TCR, T-cell receptor; mAb, monoclonal antibody.

¶To whom reprint requests should be addressed, Department of Oral Biology, University of Alabama at Birmingham.

discontinuous Percoll gradient (20, 40, and 75%) centrifugation, cells layered between 20% and 40% Percoll were collected as epithelial cells. To obtain highly enriched epithelial cells, this fraction of cells was stained with fluorescein isothiocyanate-conjugated anti-CD4 (G.K 1.5) and anti-CD8 (53–6.72; PharMingen) and then subjected to flow cytometry. The nonlymphoid cell fraction was collected as highly enriched epithelial cells with >96% cell viability. These epithelial cells expressed cytoplasmic cytokeratin and alkaline phosphatase.

Analysis of Cytokine Receptors. To characterize IL-2R and IL-7R expression, γδ T cells were incubated with biotinylated mAb anti-IL-2Rα (PC61 5.3; American Type Culture Collection) (25) or biotin-conjugated murine IL-7 (R & D Systems) followed by phycoerythrin–avidin for the analysis of IL-2R and IL-7R by flow cytometry. In some experiments, these γδ T cells were also reacted with mAb anti-IL-7R (A7R34) (26) followed by phycoerythrin-conjugated mAb MAR 18.5.

Culture Conditions for T-Cell Proliferative Responses. γδ T cells isolated from IELs (see above) were resuspended in complete RPMI 1640 medium supplemented with nonessential amino acids, sodium pyruvate, Hepes, L-glutamine, penicillin, streptomycin, gentamycin, 2-mercaptoethanol (5×10^{-5} M), and 10% fetal calf serum (5, 6, 21, 22). T cells (3×10^5/well) were then cultured in 96-well flat-bottom plates (Corning Cell Wells) in the presence or absence of an optimal concentration of recombinant murine (rm)IL-2 (100 units/ml; ×63–Ag8–653× 2) (27) and/or rmIL-7 (5 ng/ml; R & D Systems) for 24–72 hr at 37°C in an atmosphere of 10% CO_2. During the last 18 hr of incubation, 0.5 μCi (1 Ci = 37 GBq) of tritiated thymidine per well was added, the cells were harvested, and the amount of incorporation was determined by scintillation counting.

Detection of Cytokine and Cytokine Receptor-Specific mRNA. For the detection of IL-2R- and IL-7R-specific mRNA in the γδ T-cell subsets isolated from IELs, a modified, standard reverse transcriptase (RT)–PCR amplification protocol was used. Details of this procedure were extensively described in our previous studies (22, 23). Primers specific for murine IL-2Rα (5′-ATG GAG CCA CGC TTG CTG ATG TTG-3′ and 5′-CCA TTG TGA GCA CAA ATG TCT CCG-3′), IL-7R (5′-CGA GTG AAA TGC CTA ACT C-3′ and 5′-GCG TCC AGT TGC TTT CAC-3′), and β-actin (5′-TGG AAT CCT GTG GCA TCC ATG AAA C-3′ and 5′-TAA AAC GCA GCT CAG TAA CAG TCC G-3′) were obtained from Clontech or prepared by the University of Alabama at Birmingham Cytokine Core Facility. In some experiments, the expression of IL-2- and IL-7-specific mRNA by αβ IEL and intestinal epithelial cells was also examined by cytokine-specific RT–PCR. Murine IL-2 (5′-ATG TAC AGC ATG CAG CTC GCA TC-3′ and 5′-GGC TTG TTG AGA TGA TGC TTT GAC A-3′) and IL-7 (5′-GCC TGT CAC ATC ATC TGA GTG CC-3′ and 5′-CAG GAG GCA TCC AGG AAC TTC TG-3′) from Clontech were used in this study. For the amplification of IL-2, IL-7, and IL-2Rα cDNA, the program of 45 sec at 94°C, 45 sec at 60°C, and 2 min at 72°C was performed. IL-7R-specific cDNA was amplified by the program of 1 min at 94°C, 1 min at 56°C, and 30 sec at 72°C.

Quantitative RT–PCR for Cytokine Receptors. For the quantitation of the cytokine receptor-specific message, a quantitative RT–PCR was adapted by using recombinant internal standards (28–30). For the preparation of recombinant DNA standards for both IL-2R and IL-7R, two oligomers for the spacer gene (186 and 366 bp) from the restriction enzymes (*Ssp* I and *Rma* I, respectively) digested pGEM T Vector (Promega) forward and reverse primers were initially synthesized. The forward primer consisted of the target mRNA-specific 5′ primer as described above and the 5′ primer for the spacer gene. The reverse primer contained sequences for the spacer gene and 3′ cytokine receptor-specific primer as described above. To connect a specific primer and a spacer gene, the PCR procedure was conducted according to the method described in our previous study (30). These individual cytokine receptor-specific recombinant DNAs were used as a standard for the quantitation of IL-2R (470 bp) and IL-7R (222 bp) specific mRNA.

For the quantitative competitive RT–PCR, total RNA was extracted from different T-cell subsets as described (22, 23, 30). Modified competitive RT–PCR was performed according to the method described previously (28–30). Reverse transcripts of the sample RNA to cDNA was performed as described (22, 23, 30). For individual samples, several aliquots of transcribed DNA were prepared, and a series of dilutions of recombinant DNA internal standard were spiked into these aliquoted cDNA samples for subsequent PCR (28, 29). Following 35 cycles of reaction, the PCR products were electrophoresed on a 2% agarose gel and visualized by ethidium bromide (0.5 μg/ml) staining. To determine the amount of target gene mRNA (molecules/pg of total RNA), the gel was analyzed by computer imaging using a Macintosh Quadra 900 with the accompanying software (IMAGE FREEWARE from Wayne Rasband, National Institutes of Health). Thus, the equivalent target gene mRNA level present in the RNA sample was elucidated from the known amount of recombinant DNA concentration that matched that of the target mRNA PCR product.

RESULTS

Two Subsets of γδ T Cells in IELs. γδ T cells isolated from murine intestinal IELs consist of two subsets according to the density of γδ TCR expression (Fig. 1). When purified $CD3^+$ T cells from IELs of C3H/HeN mice ($H\text{-}2^k$) were analyzed for the expression of γδ TCR by flow cytometry using fluorescein-conjugated mAb anti-γδ TCR (GL3), two distinct populations of γδ T cells were observed (Fig. 1). Forty to 50% of $CD3^+$ T cells were γδ T cells and contained approximately equal frequencies of $\gamma\delta^{Dim}$ (mean intensity, 333 ± 22) and $\gamma\delta^{Bright}$ (mean intensity, 702 ± 45) T cells. This finding is consistent with a previous finding that mice of $H\text{-}2^k$ background harbored a similar number of $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells (31).

Expression of IL-2R and IL-7R by γδ T Cells. To characterize these two subsets of γδ T cells, $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells were purified by flow cytometry. An aliquot of $\gamma\delta^{Dim}$ or $\gamma\delta^{Bright}$ T cells was then examined for the expression of IL-2 and IL-7 cytokine-specific receptors. When the expression of these cytokine receptors was examined in $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ IELs, $\gamma\delta^{Dim}$ T cells expressed low levels of both IL-2R or IL-7R (Fig. 1). In contrast, $\gamma\delta^{Bright}$ T cells did not express receptors for IL-2 or IL-7. This result was also supported by the analysis of mRNA expression for IL-2R and IL-7R using RT–PCR. When RNA was isolated from other aliquots of $\gamma\delta^{Dim}$ or $\gamma\delta^{Bright}$ T cells and examined for the respective cytokine receptor-specific PCR product, 700 bp and 302 bp of amplified message, which corresponded to IL-2R and IL-7R, respectively, were found only in the $\gamma\delta^{Dim}$ T cells (Fig. 1). On the other hand, neither IL-2R nor IL-7R mRNA was detected in RNA preparations obtained from $\gamma\delta^{Bright}$ T cells. These results show that $\gamma\delta^{Dim}$ IELs constitutively express both IL-2R and IL-7R, whereas $\gamma\delta^{Bright}$ T cells do not harbor either receptor.

IL-2 and IL-7 Induce DNA Replication in γδ T Cells. It was important to examine whether $\gamma\delta^{Dim}$ T cells respond to exogenous IL-2 and/or IL-7 because these IELs express these cytokine-specific receptors *in situ* (Fig. 1). When $\gamma\delta^{Dim}$ T cells were incubated with an optimal concentration of IL-2 (100 units/ml) or IL-7 (5 ng/ml) for 24–72 hr, high levels of DNA replication (stimulation index ≈80–100) were noted following 48–72 hr of incubation (Fig. 2). Further, cocultivation of IL-2 and IL-7 provided a synergistic effect for proliferation of $\gamma\delta^{Dim}$ IELs where a stimulation index of 400 was seen. In contrast to the $\gamma\delta^{Dim}$ T-cell fraction, $\gamma\delta^{Bright}$ T cells did not respond to either IL-2 or IL-7 (Fig. 2). These findings indicated that $\gamma\delta^{Dim}$

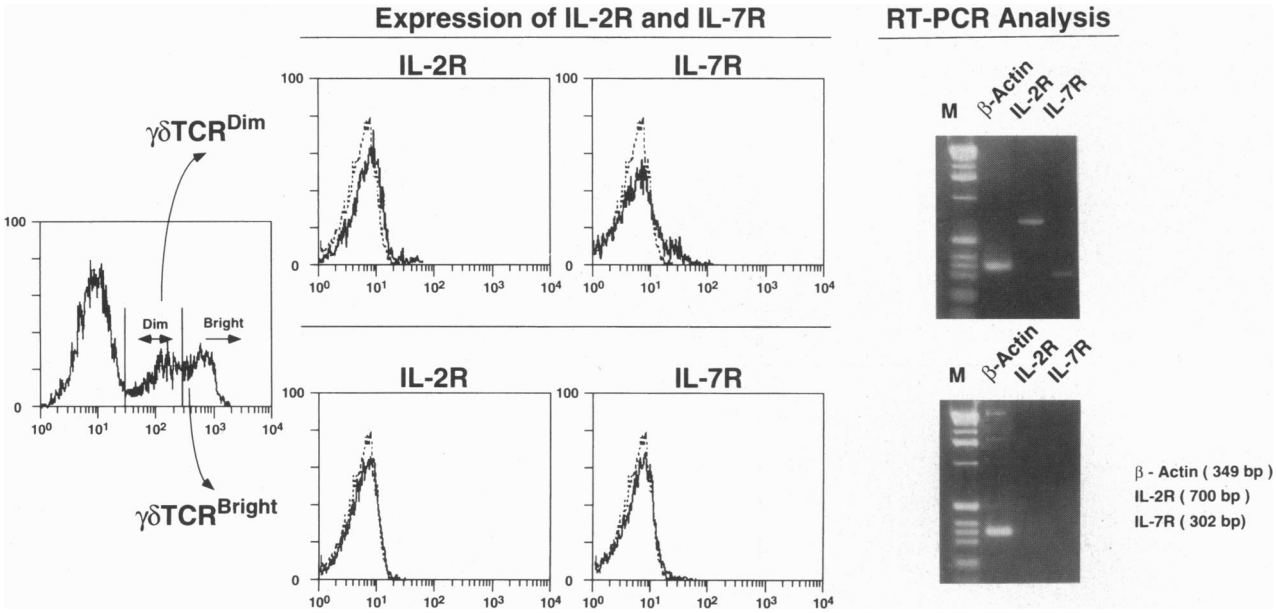

FIG. 1. Expression of IL-2R and IL-7R on γδ T-cell subsets isolated from IELs. γδ T cells isolated from murine intestinal IELs were separated into two fractions based on the intensity of γδ TCR expression (e.g., $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells). Flow cytometry analysis revealed that the $\gamma\delta^{Dim}$ T cells express both IL-2R and IL-7R whereas the $\gamma\delta^{Bright}$ T cells did not. When RNA was isolated from fluorescence-activated cell sorter purified $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells and then examined by the respective cytokine-specific RT–PCR, 700-bp and 302-bp messages, which correspond to IL-2R and IL-7R, were detected only in the $\gamma\delta^{Dim}$ T cells.

T cells in IELs that express IL-2R and IL-7R respond to exogenous IL-2 and IL-7 and lead to the high level of DNA replication and cell proliferation.

Induction of IL-2R and IL-7R on γδ T Cells. To this point, our results suggested that $\gamma\delta^{Dim}$ T cells express low levels of IL-2R and IL-7R *in situ* and both IL-2 and IL-7 are required for the induction of maximum proliferative responses. Thus, it was important to determine if IL-2 induces its own receptor or IL-7R and similarly if IL-7 affects it own receptor or influences IL-2R expression. Since maximum T-cell proliferative responses were seen after 48–72 hr of culture, the expression of IL-7R was examined following cultivation with IL-2 for the optimal incubation period (Fig. 3). High expression of IL-7R was noted on IL-2-treated $\gamma\delta^{Dim}$ T cells, but not $\gamma\delta^{Bright}$ T cells. Further, IL-2R expression was not altered by this treatment. In related experiments, IL-7 treatment induced IL-2R expression (Fig. 3), but not IL-7R on $\gamma\delta^{Dim}$ T cells. The effect of IL-7 was not seen on $\gamma\delta^{Bright}$ T cells. These experiments suggested that IL-2 can provide an appropriate stimulation signal for the upregulation of IL-7R whereas IL-7 enhances IL-2R expression on a subset of γδ T cells in IELs.

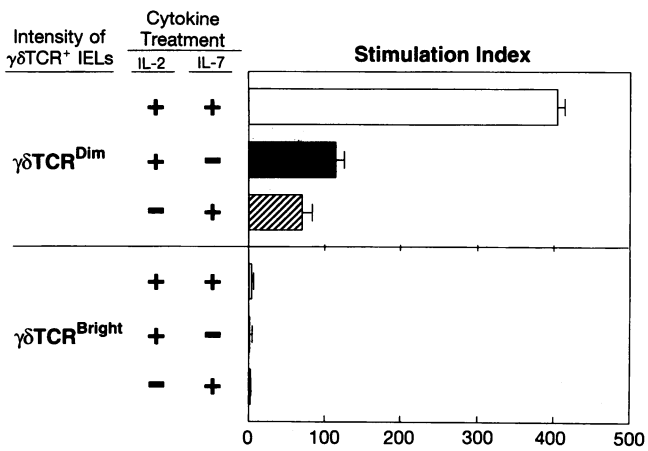

FIG. 2. Synergistic effect of IL-2 and IL-7 for the induction of high DNA replication in IL-2R and IL-7R expressing $\gamma\delta^{Dim}$ IELs. $\gamma\delta^{Dim}$ and $\gamma\delta^{Bright}$ T cells were isolated from IELs by flow cytometry. When these γδ T cells were incubated with IL-2 and/or IL-7 for 24–72 hr, the former subset resulted in high proliferative responses following 48–72 hr of cultivation, especially in the presence of both cytokines. In contrast, the later fraction did not respond to IL-2 or IL-7. The levels of [^{3}H]thymidine incorporation for control cultures (e.g., cells only) were 241 ± 36 cpm and 375 ± 121 cpm for dim and bright γδ T cells, respectively. Results represent the values (mean ± SEM) from four separate experiments (triplicate wells/experiment).

To directly prove that IL-2 and IL-7 reciprocally induced IL-7R and IL-2R at the mRNA level, a quantitative RT–PCR was performed for RNA samples obtained from IL-2- or IL-7-treated γδ T cells following 48 hr of culture. Thus, message for IL-7R was markedly increased in RNA isolated from $\gamma\delta^{Dim}$ T cells incubated with IL-2 (888.0 ± 280.3 molecules/pg of total RNA) when compared with untreated controls (40.8 ± 12.6 molecules/pg of total RNA) (Fig. 4). In the case of IL-2R expression, RNA obtained from IL-2 treated $\gamma\delta^{Dim}$ T cells contained the same or slightly higher levels of IL-2R message (20.3 ± 1.9 molecules/pg of total RNA) than untreated controls (IL-2R mRNA not detected). When RNA isolated from IL-7-treated $\gamma\delta^{Dim}$ T cells was examined, a quantitative RT–PCR analysis indicated that higher levels of IL-2R message (9684.8 ± 2801.2 molecules/pg of total RNA) were induced in IL-7 treated $\gamma\delta^{Dim}$ T cells than untreated controls (not detected) after 48 hr of incubation (Fig. 4). However, the intensity of the band for IL-7R slightly increased in the IL-7 treated $\gamma\delta^{Dim}$ IELs. Therefore, 140.9 ± 13.4 molecules/pg of total RNA of IL-7R-specific mRNA were noted in RNA from IL-7 treated $\gamma\delta^{Dim}$ T cells, while 40.8 ± 12.6 molecules/pg of total RNA of IL-7R-specific message were seen in untreated controls (Fig. 4). These findings showed that IL-2 signals by means of its own receptor on $\gamma\delta^{Dim}$ IELs, which leads to the enhancement of IL-7R expression, whereas IL-7 can elicit IL-2R-specific mRNA and receptor expression. However, $\gamma\delta^{Bright}$ T cells did not respond to either IL-2 or IL-7.

Source of IL-2 and IL-7 for $\gamma\delta^{Dim}$ IEL Stimulation. Thus far, our results demonstrated that cytokines such as IL-2 and IL-7 are key activation and growth factors for $\gamma\delta^{Dim}$ IELs. To identify the source of these cytokine-producing cells in the

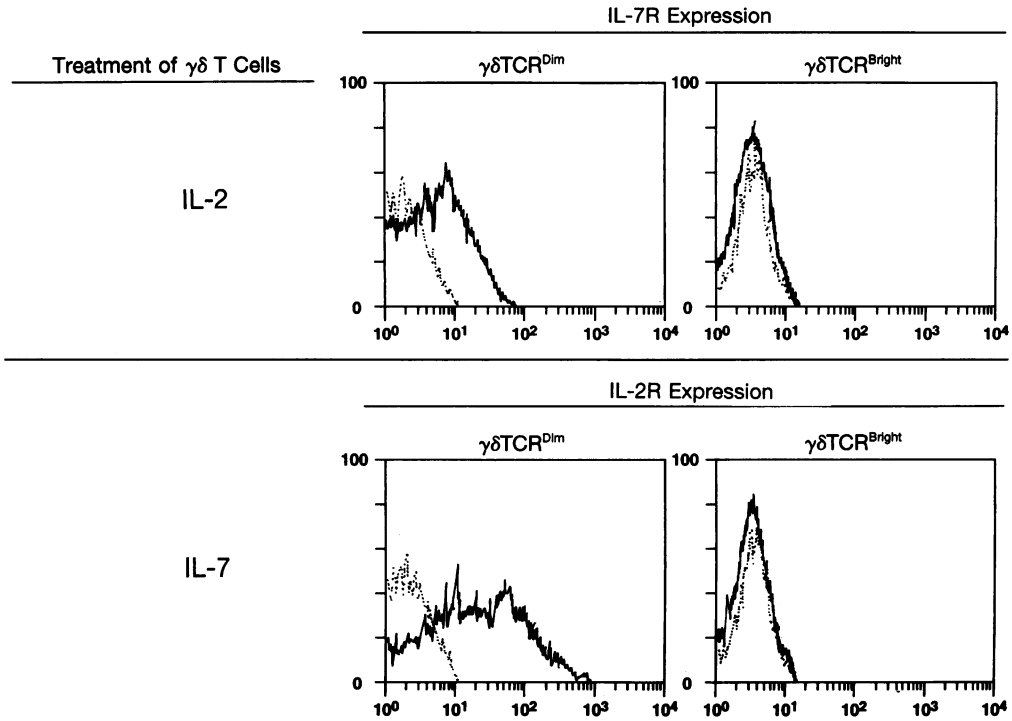

FIG. 3. Induction of IL-2R and IL-7R on $\gamma\delta^{Dim}$ IELs by IL-7 and IL-2, respectively. $\gamma\delta^{Dim}$ T cells and $\gamma\delta^{Bright}$ T cells were treated with IL-2 and/or IL-7 for 48–72 hr. The expression of IL-7R and IL-2R was then examined by flow cytometry analysis. High levels of expression of IL-2R and IL-7R were noted following incubation of $\gamma\delta^{Dim}$ T cells with IL-7 and IL-2, respectively. However, $\gamma\delta^{Bright}$ T cells, when treated in this manner, did not change IL-2R or IL-7R expression.

intestinal epithelium, $\alpha\beta$ T cells and epithelial cells were isolated from the small intestine of the same mice for the analysis of IL-2- and IL-7-specific mRNA expression. Cytokine-specific RT–PCR analysis revealed that epithelial cells harbored mRNA for IL-7 but not IL-2 (Fig. 5). As controls, type β transforming growth factor expression was examined in RNA isolated from the same preparations, and its presence was confirmed (data not shown). In the case of $\alpha\beta$ T cells, mRNA for both IL-2 and IL-7 were noted by cytokine-specific RT–PCR analysis (Fig. 5). However, the intensity of the PCR product was higher for IL-7 when compared with IL-2. Taken together, these findings suggested that both epithelial cells and $\alpha\beta$ T cells are a source of IL-7 for neighboring $\gamma\delta$ T cells. Further, $\alpha\beta$ T cells can provide IL-2 for growth and activation of $\gamma\delta^{Dim}$ IELs.

DISCUSSION

Although IL-7 was originally described as a pre-B-cell growth factor, numerous recent studies have shown that this is an important cytokine for activation, growth, and proliferation of T cells (11–21). Together with the classical T-cell growth factor IL-2, this cytokine is able to provide stimulation signals for both mature and immature T cells (14–21). The present study provided additional evidence that IL-7 could be an important cytokine for a subset of $\gamma\delta$ T cells (e.g., $\gamma\delta^{Dim}$ T cells) in the mucosal epithelium. Thus, $\gamma\delta^{Dim}$ IELs express low levels of IL-7R (Fig. 1) and respond to exogenous IL-7 (Fig. 2). This cytokine can induce DNA replication in $\gamma\delta^{Dim}$ T cells without any costimulant. Further, IL-2 synergistically enhances IL-7-induced $\gamma\delta^{Dim}$ T-cell proliferative responses (Fig. 2). Our results support the concept that IL-7 could be an important T-cell growth factor for $\gamma\delta$ T cells. To this end, IL-7 has been shown to induce proliferation of $CD8^+$, $\gamma\delta$ T cells in fetal thymocyte cultures (32, 33). It was also shown that IL-7 induces proliferative responses in $\gamma\delta$ thymocytes (20).

Our results further illustrate that IL-7 can elicit low expression of IL-2R to high levels on $\gamma\delta^{Dim}$ T cells but not on $\gamma\delta^{Bright}$ IELs (Figs. 3 and 4), and adds to earlier work which has shown that this cytokine induced IL-2R on activated T cells isolated from lymph nodes (14, 34). Our separate studies revealed that IL-7 can induce IL-2R on $\alpha\beta$ T cells, including a subset of $\alpha\beta$ T cells in IELs (data not shown). Further, IL-7 induced IL-2R expression on thymocytes (17, 18) as well as on LAK cells (35). Therefore, IL-7 may be considered an essential cytokine for the expression of IL-2R on T cells. On the other hand, IL-2 can upregulate expression of IL-7R on $\gamma\delta^{Dim}$ T cells. Incubation of $\gamma\delta^{Dim}$ T cells from murine intestinal epithelium with an optimal concentration of IL-2 resulted in the enhancement of IL-7R-specific mRNA as well as receptor expression on the cell surface (Figs. 3 and 4). This reciprocal regulation of IL-2R and IL-7R expression on $\gamma\delta^{Dim}$ T cells by IL-7 and IL-2, respectively, suggests that both receptors may share a common receptor chain. To this end, it was demonstrated that the γ_c chain is a shared component between IL-2R and IL-7R (36, 37). The γ_c-chain portion of IL-2R is a functionally essential second subunit for IL-7R to form high affinity receptor (36, 37). Taken together, it is possible that reciprocal regulation of IL-2R and IL-7R expression by IL-7 and IL-2 could be mediated via the common γ_c chain. Our recent and separate study showed that mRNA for the γ_c chain of IL-2R was expressed in $\gamma\delta^{Dim}$ T cells isolated from IELs (unpublished data).

In the present study, we have used IL-2Rα-chain-specific primer and mAb for the elucidation of receptor expression on $\gamma\delta$ IELs. It was shown that IL-2Rα expression was only seen on $\gamma\delta^{Dim}$ T cells but not $\gamma\delta^{Bright}$ T cells (Figs. 1 and 2). Further, the expression of IL-2Rα was upregulated by IL-7 (Figs. 3 and 4). In contrast to the α-chain expression, our recent and separate study showed that β chain of IL-2R was expressed on both subsets of $\gamma\delta$ T cells (data not shown). In addition, IL-2R β-chain-specific mRNA expression was not altered by the treatment of $\gamma\delta$ T cells with IL-7. According to these findings, one can postulate that epithelial cell- and/or $\alpha\beta$ T cell-derived IL-7 can provide up-

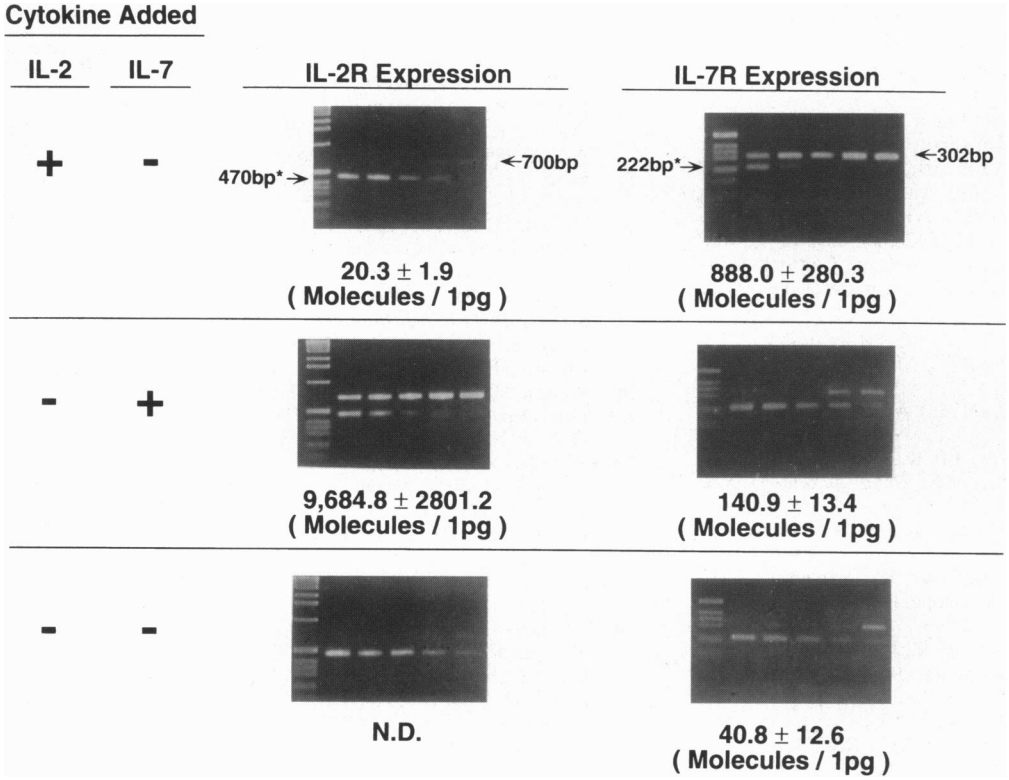

FIG. 4. Quantitative analysis of mRNA expression for IL-2R and IL-7R on IEL $\gamma\delta^{Dim}$ T cells. To quantitatively determine the expression of IL-2R and IL-7R mRNA in $\gamma\delta^{Dim}$ IELs, a quantitative RT-PCR was used. IL-2 treatment induced 888 molecules/pg of total RNA of IL-7R (302 bp) specific mRNA in $\gamma\delta^{Dim}$ T cells. Untreated $\gamma\delta^{Dim}$ T cells harbored 40.8 molecules of IL-7R-specific mRNA/pg of total RNA. Further, incubation of $\gamma\delta^{Dim}$ T cells with IL-7 resulted in 9648 molecules/pg of total RNA for IL-2R-specific mRNA (700 bp), whereas untreated cells contained undetectable levels of receptor-specific message. The asterisk indicates internal standards for IL-2R (470 bp) and IL-7R (222 bp). N.D. indicates not detectable because the intensity of the band was out of range for the measurement of specific message by the RT–PCR using internal standard for IL-2R (10–1000 amol/ml), although a weak PCR product is seen.

regulatory signals for the enhancement of IL-2R α-chain expression by means of the γc chain on $\gamma\delta$ T cells.

The results obtained by the present investigation demonstrated that IL-2 and IL-7 are essential cytokines for the activation of $\gamma\delta^{Dim}$ T cells in the intestinal epithelium. However, an important question regarding the source of these two cytokines in mucosa-associated tissues remain unanswered. One possible source of IL-7 for these $\gamma\delta^{Dim}$ T cells in the intestinal epithelium would be the epithelial cells themselves. In this regard, mRNA from thymus contained high levels of message for IL-7 (12). Further, it was suggested that the sources of the IL-7-specific mRNA could be thymic epithelial cells and/or thymic stromal cells because the production of this cytokine by thymocytes was equivocal (13, 19). Our present study verified that murine intestinal epithelial cells express IL-7-specific mRNA (Fig. 5). It was recently shown that human epithelial cells were also capable of producing IL-7 for the regulation of mucosal T cells (38).

From this discussion, we now suggest that cell-to-cell interactions between epithelial cells and $\gamma\delta^{Dim}$ IELs via IL-7 and IL-7R, respectively, could be an important cytokine communication for the activation of $\gamma\delta$ T cells in the intestinal epithelium. Thus, IL-7 produced by epithelial cells may induce DNA replication and IL-2R expression on $\gamma\delta^{Dim}$ T cells. In addition, $\alpha\beta$ T cells isolated from the intestinal epithelium could also be another source of IL-7 because RNA isolated from $\alpha\beta$ IELs harbored message for IL-7 (Fig. 5). IL-2 produced by neighboring $\alpha\beta$ T cells in IELs would further augment IL-7-induced proliferation of $\gamma\delta^{Dim}$ T cells as well as enhance the expression of IL-7R. To this end, our results showed that although the intensity of the RT–PCR product was weak, message for IL-2 was detected in $\alpha\beta$ T cells (Fig. 5). Further, our previous study indicated that $CD4^+$ $\alpha\beta$ IELs are capable of producing IL-2 upon stimulation via the TCR–CD3 complex (23). Taken together, our results suggest a triad of cell interactions among $\gamma\delta^{Dim}$ T cells, epithelial cells, and $\alpha\beta$ T cells through IL-2R and IL-7R expression and IL-7 and IL-2 synthesis, respectively, for the stimulation and development of $\gamma\delta^{Dim}$ T cells in the intestinal epithelium. In this regard, reduction in both numbers of intestinal epithelial cells and levels of major histocompatibility complex class II expression was noted in TCRδ gene disrupted mice (39). It would be interesting to examine whether production of IL-7 by epithelial cells was reduced in these TCRδ knockout mice.

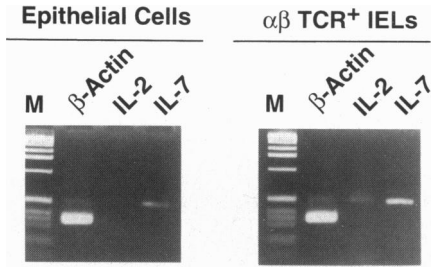

FIG. 5. Expression of IL-2- and/or IL-7-specific mRNA by intestinal epithelial cells and $\alpha\beta$ IELs. The RNAs from freshly isolated epithelial cells and $\alpha\beta$ T cells of intestinal epithelium were examined by IL-2- and IL-7-specific RT–PCR. Epithelial cells expressed mRNA for IL-7 (469 bp) but not IL-2 (502 bp), whereas $\alpha\beta$ T cells harbored both messages.

Two interesting possibilities for the life cycle of $\gamma\delta$ IELs should be discussed. The first possibility is that a shift from $\gamma\delta^{Dim}$ T cells to $\gamma\delta^{Bright}$ T cells may occur upon appropriate cytokine signals provided by the epithelial cells and $\alpha\beta$ T cells. Thus, $\gamma\delta^{Dim}$ IELs become fully activated functional cells in the presence of IL-2 and IL-7 produced by neighboring $CD4^+$ $\alpha\beta$ T cells, and epithelial cells, respectively. When these cells reach the stage of $\gamma\delta^{Bright}$ T cells, the loss of IL-2R and IL-7R occurs. Since our preliminary results indicated that neither IL-2 nor IL-7 treatment affected the intensity of TCR expression on $\gamma\delta^{Dim}$ T cells, other cytokines and antigen stimulation signals may also be involved in the shift of $\gamma\delta^{Dim}$ to $\gamma\delta^{Bright}$ expression. Alternatively, it is possible that $\gamma\delta^{Bright}$ T cells could be immature stages of T-cell development, much like the B-cell maturation process where higher levels of immunoglobulin expression are noted in B cells in an early developmental stage rather than in mature B cells. Thus, if optimal stimulation

signals were provided to $\gamma\delta^{Bright}$ T cells via the TCR–CD3 complex, the expression of IL-2R and IL-7R may be induced on these IELs which then shift to $\gamma\delta^{Dim}$ T cells.

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