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Analysis of cytokine profile in human colonic mucosal FcεRI-positive cells by single cell PCR: inhibition of IL-3 expression in steroid-treated IBD patients

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Abstract **Mast cells can serve as a possible important source of cytokine production in inflamed tissue which can be regulated by stimuli different from those activating other immune system cells. To study the expression of specific genes in mast cells derived from small human colonic mucosal endoscopic biopsies, we first modified a previously reported procedure to achieve a significantly enriched mast cell fraction. Then, by using single-cell RT-PCR analysis the expression of the IgE Fc receptor (FcεRI) and c-kit mRNA was determined. It was observed that the FcεRI-positive cells also expressed c-kit. This observation provided further evidence that FcεRI-positive cells are indeed mast cells. Analysis of biopsies from 12 patients (four control and eight patients with inflammatory bowel disease (IBD)) was carried out, revealing that all of the FcεRI-positive cells expressed IL-3, while the expression of IL-4 was detected only in some of these positive cells. TNFα was not detected in these cells. Therefore, it would seem that most intestinal mast cells produce IL-3. Since it has been reported that IL-3 synthesis was down-regulated in steroid-treated cells, the expression pattern of IL-3 in intestinal mast cells derived from steroid-treated IBD patients was then determined. IL-3 mRNA was detected in only two out of 24 FcεRI-positive cells derived from these steroid-treated patients. These results lend strong support to the idea that the down-regulation of IL-3 in mast cells derived from steroid-treated IBD patients occurs in vivo and could be an important mechanism for immunomodulation in IBD.**

Key words: Mast cell; Cytokine; Single cell RT-PCR; Corticosteroid

1. Introduction

The gastrointestinal (GI) immune system is being constantly exposed to massive antigenic stimuli and this interaction initiates humoral and cellular responses. Since cytokines were implicated in having a vital influence on the regulation, maturation, activation, proliferation and specific functions of the immune cells, they may also operate in the regulation of the immune responses in the GI tract [1–3]. However, essential information regarding their role in the immuno-regulation and inflammation of the human gut is limited. Furthermore, different stimuli induce the production of cytokines in different immune cells. For example, different enhancers are used for IL-4 expression in mast cells and in T-cells [4]. Therefore, one cannot readily deduce from the regulation of a gene in the one cell type that it is regulated similarly in other cell types.

A possible important site of cytokine production in inflamed tissue are mast cells, in which cytokine production is regulated by different stimuli than in T-cells. Mast cells which are characterized by their high-affinity Fc receptor (FcεRI), have been postulated to operate in concert with other cells in immune and inflammatory responses in the gut [5–7]. These cells are capable of synthesizing and releasing histamine, cytokines, lipid-derived compounds, proteases, endothelines growth and angiogenic factors [3,7–12].

IL-3 plays a major role in the differentiation and growth regulation of a variety of hematopoietic cell types including basophils and mast cells [3]. Recently, it was reported that cyclosporine, a potent drug for the treatment of refractory cases of inflammatory bowel disease (IBD), significantly decreases the half-life of IL-3 mRNA in vitro [13]. Furthermore, glucocorticoids, which are widely used for treatment of IBD, were shown to significantly reduce the levels of IL-3 in T-cells from asthmatic patients [14]. Since mast cells might produce a significant amount of IL-3, the possible down-regulation of this cytokine production might be an important common target of immunomodulatory drugs in IBD. We therefore determined the expression of cytokine in mast cells from normal, inflamed and steroid-treated intestinal tissue. TNFα was studied in relation to IBD as a pro-inflammatory factor [2,4,5]. IL-3 functions as a differentiating factor for immature T-lymphocytes and also promotes and maintains basophilic and mast cell growth in vitro [3]. IL-4 has a major role in the control of allergic inflammation, since not only can it synergize with other cutokines to cause increased proliferation of mast cells, it is also essential for the isotope switching in B-cells from IgG to IgE [11]. Expression of this cytokine has been reported in human basophils, bone marrow-derived mast cells and in lung mast cells from allergic patients [15,16].

IL-8 is a member of the chemokine family of cytokines, which are small proteins with several important roles in the inflammatory response [17]. It is produced by a variety of cell types and was recently shown to be produced in the human mast cell line, HMC-1, and in skin mast cells [18].

In the current work we present an approach for studying cytokine production by human colonic FcεRI-positive cells. These cells, which were derived from endoscopic mucosal biopsies, were examined for cytokine mRNA accumulation by using the single-cell polymerase chain reaction.

Previously, we have used the single-cell RT-PCR approach to analyse semiquantitavely the expression of various genes in mast cells [19] and in FcεRI-positive cells derived from human endoscopic mucosal biopsies [20]. Here, we utilized the same

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approach to obtain semiquantitative data regarding the expression of IL-3, IL-4 and IL-8 in FcεRI-positive cells derived from biopsies of IBD patients.

2. Materials and methods

2.1. Isolation and enrichment of human colonic mast cells

Mucosal specimens (weight 15–25 mg/sample) were obtained during colonoscopy performed for diagnosis and disease follow-up. Specimens were taken from normal mucosa as well as mucosa from IBD patients who had either been treated with glucocorticoids (steroids) or had not received any steroid treatment. The study was approved by the local hospital Helsinki committee. The samples were incubated in saline overnight at 4°C prior to the digestion procedure. After cleaning the mucus, the tissue was incubated in RPMI-1640 medium supplemented with 25 mM HEPES and 1 mM dithiothreitol [21]. Then, in order to separate the cells from the tissue, the tissue was sequentially treated with 30 U of collagenase (Sigma, Israel) at 37°C for 45 min and the cell suspension was filtered through a nylon wool column. The percentage of the mast cells was determined in the cell eluate by toluidine blue staining. In order to separate single cells, serial dilution of the cells was performed in a Terasaki microtiter plate until single cells were visualized in each well by inverted microscope. Each single cell was suspended in 10 μl of HBSS medium supplemented with 25 mM HEPES and incubated for 6 h in 5% CO_2 at 37°C.

2.2. Preparation of RNA

Each cell was transferred from its well into a 1.5-ml Eppendorf tube containing 100 μl of lysis buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA and 0.5% SDS) supplemented with 100 μg proteinase K and incubated for 10 min at 37°C. This was followed by the addition of 18 μl of 3 M NaCl and 50 μg oligo-dT cellulose. The mixture was incubated overnight by rotation at room temperature. The oligo-dT cellulose was washed 3 times with binding buffer (0.5 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA and 0.1% SDS) followed by one wash with washing buffer (0.1 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA and 0.1% SDS). The RNA was eluted by incubating the oligo-dT for 10 min at room temperature with 50 μl of 10 mM Tris-HCl, pH 8.3. The mRNA was ethanol-precipitated in the presence of 5 μg tRNA, spun and then washed with 70% ethanol. The dried RNA pellet was redissolved in RT buffer containing RNase inhibitor [19,20].

2.3. Oligonucleotide probes and primers

cDNA probes for human αFcεRI (kindly provided by Dr. J.P Kinet, National Institutes of Health, Bethesda, MD), human IL-3 (kindly provided by the Genetic Institute, Cambridge, MA), human IL-4 (provided by DNAX, Palo Alto, CA), human IL-8 (kindly provided by Dr. Matsushuma, National Cancer Institute, Frederic, MD), human c-kit (kindly provided by A. Bernstein, Toronto, Canada) and for human TNFα the PCR amplified fragment from RNA derived from human peripheral blood leukocytes was used as a probe. The sequence targeted by each pair of oligonucleotides was designed to span an intron/exon border in order that fragments amplified from cDNA generated from mRNA could be readily distinguished by their much smaller size from those predicted to be generated from any contaminating genomic DNA. αFcεRI oligonucleotide primers: sense: 5′-ATG GCT CCT GCC ATG GAA TCC CCT ACT-3′ and anti-sense: 5′-GGT TCC ACT GTC TTC AAC TGT GGC AAT-3′ corresponding to bases 106–519 [22]. IL-4 primers: sense: 5′-ATG GGT CTC ACC TCC CAA CTG CTT CCC-3′ and anti-sense: 5′-ATT TCT CTC TCA TGA TCG TCT TTA GCC-3′ corresponding to bases 64–505 [23]. IL-3 primers: sense: 5′-GCT CCC ATG ACC CAG ACA ACG TCC TTG-3′ and anti-sense: 5′-GTC CTT GAT ATG GAT TGG ATG TCG CGT-3′ corresponding to bases 67–360 [24]. IL-8 primers: sense: 5′-ATG ACT TCC AAG CTG GCC GTG GCT CTC-3′ and anti-sense: 5′-ATG AAT TCT CAG CCC TCT TCA AAA ACT-3′ corresponding to bases 1–295 [25]. TNFα primers: sense: 5′-CTG CGC CAG GCA ACG GGT CCT GCG CCT-3′ and anti-sense: 5′-CAG CTT CAG GTC CCT CAA AGC GCT GCG GGG-3′ [26]. c-Kit primers: sense: 5′-TCA CAG CTT GGC AGC CAG-3′ and antisense: 5′-GGG GAT CTG CAT CCC AGC AAG-3′ corresponding to 2384–2754 [27].

2.4. RT-PCR

RNA was reverse transcribed into cDNA essentially as previously described [19,20]. RNA from one single cell was incubated at 37°C for 60 min with a mixture of 400 U of MMLV-RT and the following reagents: 3 μM sequence specific antisense primers of αFcεRI and either one of the cytokines described above: 1:5 (V/V) ×5 reaction buffer, 0.5 mM dNTP and 5U of RNAsin in 20 μl of final volume. Each of the RT mixtures was added to 2.5 U of taq 1 DNA polymerase to a final volume of 100 μl containing 3 μM 5′ sequence-specific primer of αFcεRI and either one of the cytokines described above: 0.25 mM dNTP, 10 mM Tris-HCl, pH 8, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% (w/v) gelatin. The mixture was covered with mineral oil and subjected to the amplification process in a repeated three temperature cycle in a programmable thermal controller (MJ Research Inc.). The temperature used in the annealing cycle was determined according to the G–C content of the primers. The samples were amplified up to 30 cycles.

2.5. Agarose gel electrophoresis

The PCR mixture (10 μl) was added to 2 μl of loading dye electrophoresed in a 80-V constant voltage field in 1% sodium agarose until the bromophenol-blue dye front had migrated 8 cm.

2.6. Southern blot hybridization analysis

The PCR-amplified fragments separated on agarose gels were transferred to nylon hybridization transfer membranes. The membrane-bound DNA was denatured with 0.5 N NaOH. Each of the hybridization transfer membranes was hybridized with a ^{32}P-labelled probe by standard techniques and washed at 65°C for 20 min in 0.1×SSC containing 0.1% SDS prior to autoradiography.

3. Results

3.1. Isolation and enrichment of colonic mucosal mast cells

Using the procedure for isolation of intestinal mast cells [21], only 2% of the cells were found to be positively stained by toluidine blue. Therefore, this procedure was modified by the addition of overnight pre-incubation of the tissue at 4°C prior to the enzymatic digestion. This modification has been described previously for the purification of viable and well-functioning human lung mast cells [28]. By using this modification, 20–35% of the column-eluted cells were positively stained with toluidine blue.

3.2. Cytokine expression by FcεRI-positive cells

In order to determine a few of the cytokines expressed by

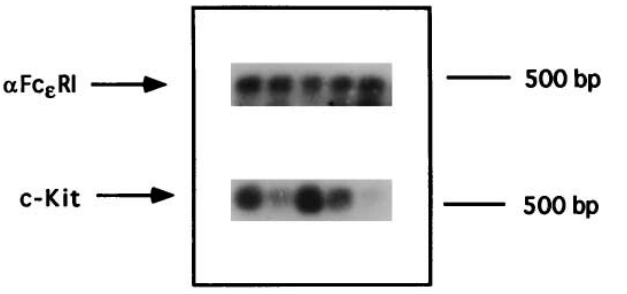

Fig. 1. Expression of c-kit in FcεRI-positive cells isolated from biopsies of human colonic mucosa. mRNA from single cells was reverse-transcribed with αFcεRI and c-kit primers and the products were PCR amplified for 30 cycles. PCR products were separated by agarose gel electrophoresis, transferred to nylon hybridization membrane, and then hybridized with ^{32}P-labeled cDNAs for αFcεRI. Only those cell-derived products that were found to be FcεRI-positive after autography were separated again by agarose gel electrophoresis, transferred to nylon hybridization membrane, and then hybridized with ^{32}P-labeled cDNA for c-kit and autoradiographed for 17 h. One representative experiment out of three is shown.

individual colonic mucosal FcεRI-positive cells, we applied the RT-PCR single-cell technique previously utilized by us and others [19,20,29]. The characterization of the PCR-amplified cDNA fragments generated by using oligonucleotides specific for FcεRI and either one of the cytokines was performed by the hybridization of the Southern blots with the specific ^{32}P-labelled cDNAs. A good correlation was found between the percentage of toluidine blue-positive cells and those expressing *FcεRI* gene. Furthermore, in order to strengthen the possibility that the majority of the FcεRI-positive cells are mast cells rather then basophils, eosinophils or monocytes three types of cells which also express surface FcεRI [30–32] we determined whether the FcεRI-positive cells also expressed c-kit as this proto-oncogene is known to be expressed in mast cells but not in basophils, eosinophils or monocytes [33]. Fig. 1 shows that all of the FcεRI-positive cells expressed the c-kit although there were differences in the levels of c-kit products in the various cells.

It was previously reported that the α-subunit of the FcεRI is consistently expressed in mast cells [34]. Thus, this transcript should be considered a reference for the various cytokine mRNAs amplified in the single cells. Fig. 2 shows a representative experiment using the single-cell RT-PCR technique to detect cytokines expressed by mucosal FcεRI-positive cells. IL-3 was expressed in all of these cells while IL-8 was expressed in two out of four cells. IL-4 was expressed in one out of four cells. Although the single-cell RT-PCR method as used here cannot determine quantitatively the level of mRNA in an individual cell, it allows us to observe, under defined PCR cycles, which of the FcεRI-positive cells express these cytokines above a certain level.

Expression of IL-3, IL-4 and IL-8 by the FcεRI-positive cells derived from normal and IBD mucosa is summarized

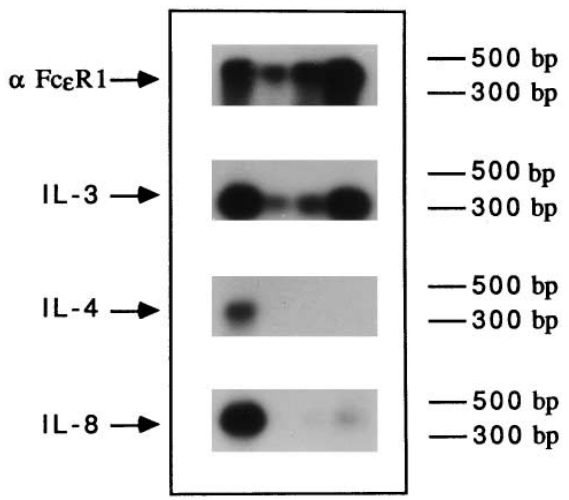

Fig. 2. Expression of IL-3, IL-4, IL-8 mRNAs in FcεRI-positive cells isolated from biopsies of human colonic mucosa. mRNA from single cells was reverse-transcribed with αFcεRI primers and primers from either one of the following cytokines: IL-3, IL-4 and IL-8. The products were PCR amplified for 30 cycles. PCR products were first hybridized with ^{32}P-labeled cDNAs for αFcεRI. Only those cell- derived products that were found to be FcεRI-positive after autoradiography were separated again by agarose gel electrophoresis, transferred to nylon hybridization membrane, and then hybridized with ^{32}P-labeled cDNAs for αFcεRI, IL-3, IL-4 and IL-8 and autoradiographed for 17 h. One representative experiment out of four is shown.

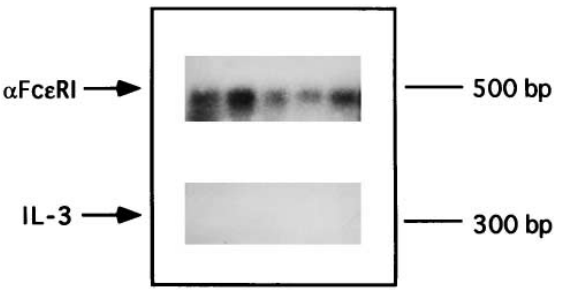

Fig. 3. Inhibition of IL-3 mRNA expression in FcεRI-positive cells isolated from biopsies of human colonic mucosa derived from steroid-treated patients. mRNA from single cells was reverse-transcribed with αFcεRI and IL-3 primers. The products were PCR amplified for 30 cycles. PCR products were first hybridized with ^{32}P-labeled cDNAs for αFcεRI. Only those cell-derived products that were found to be FcεRI-positive after autoradiography were separated again by agarose gel electrophoresis, transferred to nylon hybridization membrane, and then hybridized with ^{32}P-labeled cDNAs for IL-3 and autoradiographed for 17 h. One representative experiment out of five is shown.

in Table 1. All single cells examined are FcεRI-positive, c-kit-positive and predicted to be toluidine blue-positive. All IBD cases had active ulcerative colitis, and biopsies were taken from the inflamed mucosa. There was no significant difference in the recovery of toluidine blue-positive cells isolated from biopsies taken from IBD or control subjects. IL-3 was expressed in all FcεRI-positive cells. Interestingly, IL-8 was significantly much less expressed in FcεRI-positive cells derived from inflammatory than from normal mucosa (16% vs. 85% respectively; Table 1). Cells from the inflamed mucosa were more likely to express IL-4 as compared to FcεRI-positive cells derived from normal mucosa (38% vs. 13% in control). In repeated experiments, utilizing oligomers with which successful amplification of TNFα from peripheral blood cell mRNA has been carried out, we could not detect any TNFα from FcεRI-positive cells derived from either inflammatory or normal mucosa (results not shown).

3.3. Effect of steroid-treatment on IL-3 expression in mast cells

Since a large number of IBD patients are treated with steroids, the accumulation of IL-3 mRNA was determined in FcεRI–c-kit-positive cells isolated from human colonic mucosa of 10 steroid-treated IBD patients. There was no significant difference in the toluidine blue-positive cells isolated from biopsies taken from IBD, control or steroid-treated IBD subjects. In stark contrast to the 44 IL-3-positive cells isolated from untreated mucosal FcεRI-positive cells, only 4 IL-3 positive cells were detected out of 40 FcεRI-positive cells isolated from steroid-treated IBD patients (Table 1 and Fig. 3).

4. Discussion

Mast cells have been shown to play an important role in physiological processes as well as in inflammation and immune response [8,11,35]. They are a rich source of various biologically active mediators such as histamine, eicosanoids, platelet activating factor, cytokines, growth factors and endothelins [7,9–12] which act in the local milieu as regulators and paracrine effectors. They have also been reported to operate in immune and inflammatory responses involving the GI tract [2,3,5–7,35–37]. However, most of the data regarding the role

Table 1
Cytokine production by intestinal mucosal FcεRI-positive cells

Sample	FcεRI/total cells	IL-3/FcεRI	IL-4/FcεRI	IL-8/FcεRI
Control				
1	1/ 10	1/ 1	1/ 1	0/ 1
2	7/ 20	7/ 7	0/ 7	7/ 7
3	5/ 10	5/ 5	1/ 5	4/ 5
4	2/ 10	2/ 2	0/ 2	–
Total	15/ 50	15/15	2/15	11/13
Inflamed tissue				
1	4/ 10	4/ 4	0/ 4	0/ 4
2	5/ 10	5/ 5	1/ 5	0/ 5
3	3/ 10	3/ 3	1/ 3	3/ 3
4	1/ 10	1/ 1	0/ 1	0/ 1
5	5/ 10	5/ 5	5/ 5	0/ 5
6	3/ 10	3/ 3	1/ 3	–
7	5/ 10	5/ 5	–	–
8	3/ 10	3/ 3	–	–
Total	29/ 80	29/29	8/21	3/18*
Steroid-treated IBD patients				
1	10/ 30	0/10	–	–
2	5/ 10	2/ 5	–	–
3	2/ 10	0/ 2	–	–
4	4/ 10	0/ 4	–	–
5	3/ 10	0/ 3	–	–
6	3/ 10	0/ 3	–	–
7	4/ 10	1/ 4	–	–
8	4/ 10	0/ 4	–	–
9	2/ 10	0/ 2	–	–
10	3/ 10	1/ 3	–	–
Total	40/120	4/40	–	–

Statistical evaluation was performed according to the 2-tailed Fisher exact test (*significantly different from control $P < 0.001$).

of mast cells in the GI tract have been derived from animal studies [7]. Reports on the characterization and function of human intestinal mast cells are limited [15,21,36]. The main obstacle for studying mast cells derived from a specific organ is in obtaining a pure population of these cells. Fox and her colleagues achieved a fraction of only 6% of human colonic mucosal mast cells from isolated surgical specimens [21], and Befus and his colleagues reported on 8% mast cell purity derived from human gut [37]. Therefore, studies utilizing pure and viable human mast cells directly isolated from the human intestine have not yet been carried out. We decided to address the issue of studying pure human intestinal mast cells by a two-step approach. First, we enriched the fraction of human colonic mast cells obtained from small endoscopic mucosal biopsies up to 35% by modifying the previously reported procedure [21]. Then, by using the single-cell RT-PCR technique [19,20] only those cells which expressed FcεRI were further analyzed for their specific cytokine content. The positive correlation between the percentage of the toluidine-positive and cells expressing FcεRI, and the observation that all of the FcεRI-positive cells expressed c-kit, supported the assumption that the majority of the isolated FcεRI-positive cells were mast cells. Thus, the reported technique provides a reliable and reproducible tool for close study of the FcεRI-positive cells, probably mast cells, isolated from mucosal specimens obtained during GI endoscopic procedures.

Single-cell RT-PCR has been used for the characterization of cells which are widely dispersed between other cell types. For example, the expression pattern of several genes was determined by this method in a number of Reed-Stenberg cells from several patients [29]. A possible advantage of this method compared with in situ hybridization is the ability to detect the expression of several genes in a single defined cell. Although this method is not quantitative, by performing a limited number of PCR cycles and detection of expression of the same genes in a number of cells, semiquantitative information can be obtained if there are large differences in gene expression patterns in different situations. Previously we utilized this method to demonstrate the induction of IL-3 production in mast cell in vitro by co-culturing them with fibroblasts [19]. Therefore, the results from our study can provide general information on the expression of specific genes in intestinal FcεRI-positive cells. This, of course, could be carried out only in extreme cases where there are large differences in the expression rate of specific gene.

Since cytokines have been implicated as essential paracrine modulators in experimental colitis and IBD [1–3,38], the expression of IL-3, IL-4 and IL-8 was determined in the purified FcεRI-positive cells. Although the total number of IL-4 expressing cells was higher in FcεRI-positive cells from IBD patients, most of this increase stemmed from the results obtained from one patient and therefore cannot be considered significant. Recently Bradding et al. demonstrated by in situ hybridization that IL-4 was preferentially expressed in a subset of human bronchial mucosa mast cells [39]. Further research is needed in order to determine whether IL-4 is produced in a specific subset of mast cells in intestinal mucosa.

We also checked the expression of the pro-inflammatory cytokine IL-8 in $Fc_{\varepsilon}RI$-positive cells. This cytokine has been shown to be expressed by human mast cells [18] and also by GI epithelial cells [40].

Although IL-8 is a typical feature of mucosal inflammation, we observed that IL-8 was expressed less in $Fc_{\varepsilon}RI$-positive cells derived from inflamed as opposed to normal mucosa. However, this result is based on cells from only a few patients; therefore, further studies have to be carried out in order to elucidate the expression of this cytokine in mucosal mast cells derived from inflamed tissues. Since IL-8 is also

produced by epithelial cells, a possibility exists that its total amount is increased in inflamed tissue as a result of production of this cytokine by epethelial cells, while mast cell production of IL-8 is reduced.

We were unable to detect TNFα cDNA from $Fc_{\varepsilon}RI$-positive cells derived from inflamed or from normal mucosa. Although we used the same TNFα primers for the successful amplification of cDNA from mRNA of peripheral blood cells, our failure could be a technical one. However, in this respect it is interesting to note that immunohistochemistry studies of intestinal inflamed tissue revealed that this cytokine was most probably produced by monocytes [41].

IL-3 is one of the predominant cytokines produced by activated mast cells and has an effect on the differentiation and proliferation of several hematopoietic lineages, including those leading to the production of mast cells and basophils. Interestingly, we found that all mast cells, derived either from normal tissue or from patients with active IBD, expressed IL-3. It seems that intestinal FcεRI-positive cells produce a basal level of IL-3. A recent study has shown that glucocorticoid therapy of asthmatics is associated with a reduction in the expression of IL-3 mRNA and in the secretion of the IL-3 protein in T-cells [14]. Furthermore, cyclosporine, a potent drug in the treatment of IBD was shown to reduce mast cell IL-3 half-life in vitro. Thus, studying IL-3 expression in mucosal mast cells derived from steroid-treated IBD patients may provide an important clue as to whether this immunomodulatory mechanism occurs in mast cells in vivo. Our detection of only four expressing cells out of 40 FcεRI-positive cells derived from the mucosa of 10 steroid-treated patients is in sharp contrast to the detection of 44 positive IL-3 expressing cells out of 44 FcεRI-positive cells from untreated patients, therefore providing strong evidence in support of the notion that IL-3 expression is down-regulated in vivo in FcεRI-positive mucosal cells. Thus, mast cell IL-3 down-regulation may be one of the major targets of immunomodulatory treatment in IBD patients.

In summary, these results demonstrate the expression of several types of cytokines in intestinal FcεRI-positive cells, which are mostly mast cells. Furthermore, our demonstration of a large reduction in the number of FcεRI-positive cells expressing IL-3 from intestinal mucosa of steroid-treated patients, lends strong support to the idea that the down-regulation of IL-3 in mast cells derived from steroid-treated IBD patients occurs in vivo and could be an important mechanism for immunomodulation in IBD.

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References

[1] Fiocchi, C. (1992) Cytokines. in: MacDermott, R.P. and Stenson, W.F. (Eds.), Inflammatory Bowel Disease, Elsevier, New York.
[2] Beagley, K.W. and Elson, C.O. (1992) 21, 347–358.
[3] Elson, C.O., Kagnoff, M.F., Fiocchi, C., Befus, A.D. and Taragan, S. (1986) Gastroenterology 91, 746–760.
[4] Weiss, D.L., Hural, J., Tara, D., Timmerman, L.A., Henkel, G. and Brown, M.A. (1996) Mol. Cell. Biol. 16, 228–241.
[5] Benyon, R.C., Bissonnette, E.Y. and Befus, A.D. (1992) in: MacDermott, R.P. and Stenson, W.F. (Eds.), Inflammatory Bowel Disease, Elsevier, New York, p. 137.
[6] Ferguson, A., Cummins, A.G., Munro, G.H., Gibson, S. and Miller, H.R. (1987) Ann. Allergy 59, 40–47.
[7] Wershil, B.A. and Galli, S.J. (1991) Gastroenterol. Clin. N. Am., 613.
[8] Galli, S.J. and Lichtenstein, L.M. (1988) Biology of mast cells and basophiles, in: Middleton Jr., E., Reed, C.E. and Ellis, E.F. (Eds.), Allergy: Principles and Practice, 3rd edn., CV Mosby, St. Louis, MO, p. 106.
[9] Gordon, J.R., Bird, P.R. and Galli, S.J. (1990) Immunol. Today 11, 458–462.
[10] Plaut, M., Pierce, J.H. and Watson, C.J. (1989) Nature 339, 64–67.
[11] Paul, W.E., Seder, R.A. and Plaut, M. (1993) Adv. Immunol. 53, 147–163.
[12] Ehrenreich, H., Burd, P.R., Rottem, M., Hultner, L., Hylton, J.B. and Garfield, M. et al. (1992) New Biologist 4, 147–161.
[13] Nair, A.P., Hann, S., Banholzer, R., Hirsch, H.H. and Moroni, C. (1994) Nature 369, 239–242.
[14] Corrigan, C.J., Hamid, Q., North, J., Barkans, J., Moqbel, R., Durham, S., Gemou-Engesaeth, V. and Kay, B. (1995) Am. J. Respir. Cell. Mol. Biol. 12, 576–589.
[15] Piccinni, M.P., Macchia, D., Parronchi, P., Giudizi, M.G., Bani, D. and Alterini, R. et al. (1991) Proc. Natl. Acad. Sci. USA 88, 8656–8662.
[16] Hunt, T.C., Heusser, C., Mueller, R., Bullock, G., Okayama, Y., Ashman, L.K. et al. (1992) Aller. Clin. Immunol. 89, 245.
[17] Graves, D.T. and Jiang, Y. (1995) Crit. Rev. Oral. Biol. Med. 6, 109–120.
[18] Moller, A., Lippert, U., Lessmann, D., Kolde, G., Hamann, K. and Welker, P. et al. (1993) J. Immunol. 151, 3261–3268.
[19] Razin, E., Leslie, K.B. and Schrader, J.W. (1991) J. Immunol. 146, 981–990.
[20] Nechushtan, H., Soreq, H., Kuperstein, V., Tshori, S. and Razin, E. (1996) FEBS Lett. 379, 1–6.
[21] Fox, C.C., Dvorak, A.N., Peters, S.P., Kagey-Sobotka, A. and Lichtenstein, L.M. (1985) J. Immunol. 135, 483–495.
[22] Kochan, J., Pettine, L.F., Hakimi, J., Kischi, K. and Kinet, J.P. (1988) Nucl. Acids Res. 16, 3584–3585.
[23] Yokota, T., Otsuka, T., Mosmann, T., Banchereau, J., DeFrance, T. and Balanchard, D. et al. (1986) Proc. Natl. Acad. Sci. USA 83, 5894–5899.
[24] Yang, Y.C., Ciarrietta, A.B., Temple, P.A., Chung, M.P., Kovacic, S. and Witek-Giannotti, J.A. et al. (1986) Cell 47, 3–12.
[25] Oppenheim, J.J., Zachariae, C.O., Mukaida, N. and Matsushima, K. (1991) Annu. Rev. Immunol. 9, 617–633.
[26] Yamamura, M., Wang, X.H., Ohmen, J.D., Uyemura, K., Rea, T.H. and Bloom, R.L. et al. (1992) J. Immunol. 149, 1470–1480.
[27] Yarden, Y., Kuang, W.J. and Yang-Feng, A. (1987) EMBO J. 6, 3341–3352.
[28] Caulfield, J.P., Lewis, R.A., Hein, A. and Austen, K.F. (1980) J. Cell Biol. 85, 299–313.
[29] Trumper, L.H., Brady, C., Bagg, A., Gram, D., Loke, S.L., Griesser, H., Wagman, R., Braliel, R. and Gasloyne, R.D. et al. (1993) Blood 811, 3097–3110.
[30] Sutton, B.J. and Gould, H.J. (1993) Nature 366, 421–424.
[31] Gounni, A.S., Lamkhioued, B., Ochiai, K., Tanaka, Y., Delapote, E. and Capron, E.A. et al. (1994) Nature 367, 183–187.
[32] Maurer, D., Fiebigen, E., Wolfniniski, B., Jouvin, M.H., Kilgus, O., Kinet, J.P. and Stingl, G. (1994) J. Exp. Med. 179, 745–758.
[33] Valent, P. and Bettelhim, P. (1992) Adv. Immunol. 52, 333–350.
[34] Ra, C., Jouvin, H. and Kinet, J.P. (1989) J. Biol. Chem. 284, 15323–15330.
[35] Galli, S.J. and Austen, K.F. (1989) Raven Press, New York.
[36] Befus, A.D., Dyke, N., Goodacre, R. and Bienenstock, J. (1987) J. Immunol. 138, 2604–2614.
[37] Fox, C.C., Lazenby, A.J., Moore, W.C., Yardley, J.H., Bayless, T.M. and Lichtenstein, L.M. (1990) Gastroenterology 99, 119–130.
[38] Ihle, J.N., Rebar, L., Keller, J., Lee, J.C. and Hapel, A. (1982) Immunol. Rev. 63, 5–35.
[39] Bradding, P.Y., Okayama, P.H., Howarth, M.K., Church, M. and Holgate, S.T. (1995) J. Immun. 155, 297–310.
[40] Eckmann, L., Jung, H.C., Schurer-Maly, C., Panja, A., Morzycka- Wroblewska, E. and Kagnoff, M.E. (1993) Gastroenterology 105, 1689–1701.
[41] Cappello, M., Keshav, S., Prince, C., Jewel, D.P. and Gordon, S. (1992) Gut 33, 1214–1225.