

Nonreleasing basophils convert to releasing basophils by culturing with IL-3

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The extent of basophil histamine release initiated by IgE cross-linking stimuli has been known to vary greatly among donors. Studies on anti-IgE nonreleasing basophils are useful in understanding the IgE-specific control mechanism of mediator release. We attempted to determine (1) whether a mutation of FcεRI is present in nonreleasing basophils and (2) whether treatment with IL-3 converts anti-IgE nonreleasing basophils to releasing basophils. Basophils were purified from normal human blood and donors were divided into releasers (maximal histamine release >5%) and nonreleasers (<5%). The mutation of FcεRIα, β, and γ was evaluated by reverse transcriptase–polymerase chain reaction, and the DNA sequence was determined from amplified polymerase chain reaction products. Although antibodies against FcεRI failed to cause histamine release in anti-IgE nonreleasing basophils, no primary structural change of FcεRI was observed in nonreleaser basophils. After culturing with IL-3 for 7 days, nonreleasing basophils released histamine in response to anti-IgE, and dose-response curves of anti-IgE were equal in both releasers and nonreleasers. The conversion of nonreleasing basophils to releasing basophils was evident after 3 days of culture with IL-3. These findings indicate that nonreleasing basophils have recoverable defect(s) in the signal transduction pathway after IgE cross-linking. (J Allergy Clin Immunol 1996;97:1279-87.)

Key words: Basophils, anti-IgE, releasability, FcεRI, DNA sequence, IL-3, histamine release

Basophils are the least abundant cells in human blood, comprising only 0.5% to 1% of peripheral leukocytes. They express high-affinity receptors for IgE (FcεRI) on their surface and contain histamine in their cytoplasmic granules. After interaction of membrane-bound IgE antibodies with a specific antigen, basophils can release preformed mediators and metabolites of arachidonic acid, and they play an active role in the pathogenesis of hypersensitivity reactions.^{1, 2}

Abbreviations used

FcεRI:	High-affinity receptor for IgE
FMLP:	N-formyl-methionyl-leucyl-phenylalanine
PIPES:	Piperazine-N,N'-bis[2-ethanesulfonic acid]
RT-PCR:	Reverse transcriptase–polymerase chain reaction

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It has been widely perceived that the extent of basophil histamine release varies among donors; stimulation with a concentration of anti-IgE induces almost complete histamine release in one donor, whereas the same stimulation induces only a slight release in another donor.^{3, 4} The intrinsic property of the basophil that predetermines its ability to respond to IgE-mediated stimulation is called basophil releasability. In addition to the wide variability of histamine release among populations, basophils from 10% to 20% of all donors completely fail to release histamine in response to anti-IgE.⁵ Studies on these anti-IgE nonreleasing

basophils are useful in understanding the IgE-specific control mechanism of mediator release. As early as 1973, Ishizaka et al.⁶ demonstrated that the low response of basophils was not due to the lack of surface IgE antibodies by using passive sensitization with reaginic serum, followed by antigenic or anti-IgE stimulation. It has been well recognized that the surface IgE densities in both releasing and nonreleasing basophils are equal^{5, 7} and that anti-IgE nonreleasing basophils cause histamine release in response to non-IgE-mediated stimuli, such as 12-O-tetradecanoylphorbol 13-acetate, calcium ionophore A23187, and N-formyl-methionyl-leucyl-phenylalanine (FMLP).⁵ In addition, it has been demonstrated that nonreleasing basophils fail to show changes in the cytosolic free Ca^{2+} concentration after addition of anti-IgE.⁷ These observations indicated that the non-IgE-mediated signal transduction pathway is not impaired in anti-IgE nonreleasing basophils and suggested that the cross-linking of membrane-bound IgE does not result in transduction of an amount of intracellular signals sufficient to cause a secretory response in nonreleasing basophils. The control mechanism of basophil releasability has become better understood in recent years; however, the precise steps responsible for the lack of response remain to be clarified. In this study we attempted to determine (1) whether a mutation of Fc ϵ RI is present in nonreleasing basophils and (2) whether treatment with IL-3 converts anti-IgE nonreleasing basophils to releasing basophils.

METHODS

Reagents

The following reagents were purchased as indicated: goat anti-human IgE antibody (concentration, 36.04 mg/ml; Medical Biological Laboratory, Nagoya, Japan); goat anti-mouse IgG antibody (affinity-purified; Tago, Burlingame, Calif.); Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden); phosphate-buffered saline, fetal calf serum, and RPMI-1640 medium (Gibco, Grand Island, N.Y.); mouse anti-CD2, anti-CD14, and anti-CD19 antibodies (Becton Dickinson, Mountain View, Calif.); mouse anti-CD16 antibody (Immunotech, Marseille, France); ionophore A23187 (Calbiochem-Behring, La Jolla, Calif.); piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES) and FMLP (Sigma Chemical Co., St. Louis, Mo.). Purified human recombinant IL-3 was kindly donated by Dr. Gordon G. Wong (Genetics Institute, Cambridge, Mass.).⁸

Buffer

PIPES A buffer contained 25 mmol/L PIPES, 119 mmol/L NaCl, 5 mmol/L KCl, and 0.03% human serum

albumin and was adjusted to pH 7.4. For histamine release reactions, PIPES A containing 2 mmol/L Ca^{2+} and 0.5 mmol/L Mg^{2+} (PIPES ACM) was used.

Cell preparation

Venous blood was obtained from healthy volunteers with no history of atopic diseases such as bronchial asthma, allergic rhinitis, atopic dermatitis, and chronic urticaria after their informed consent was given.

Basophils were purified by using Percoll density centrifugation, followed by negative panning selections as described previously.⁹⁻¹¹ Briefly, venous blood was anticoagulated with ethylenediaminetetraacetic acid and overlaid on Percoll solutions of different densities (1.079 and 1.070 gm/ml). After centrifugation at 700 g for 15 minutes at room temperature, cell bands between the two Percoll solutions were collected. Cell pellets were resuspended in phosphate-buffered saline containing mouse monoclonal antibodies (anti-CD2, anti-CD14, anti-CD19, and anti-CD16) and incubated at 4° C for 1 hour. The cell suspension was washed and added to a plastic Petri dish previously coated with goat anti-mouse IgG. After incubation in an ice bath for 1 hour, the dish was gently swirled, and nonadherent cells were collected. The purity of basophils was 77% (range, 44% to 97%; $n = 15$) as determined by May-Grünwald-Giemsa staining, and the overall recovery rate was 17.2% (range, 10.6% to 51.2%; $n = 15$) as assessed by alcian blue staining. The viability of purified cells as determined by trypan blue exclusion test was consistently greater than 95%.

Histamine release

Histamine release was performed as previously described.¹² In brief, leukocytes were stimulated with the secretagogues in PIPES ACM at 37° C for 45 minutes in polystyrene tubes. The supernatants were collected by centrifugation, and released histamine was measured by using an automated fluorometric technique.¹³ Histamine release was expressed as a percentage of the total cellular histamine after subtracting the spontaneous release (consistently less than 7%) in the absence of secretagogues. Each experiment was performed at least in duplicate.

Cell culture

The complete medium consisted of RPMI-1640 medium supplemented with 300 pmol/L IL-3, 10% fetal calf serum, 2 mmol/L L-glutamine, and antibiotics (100 U of penicillin, 100 µg of streptomycin, and 250 µg of Fungizone per milliliter). Purified basophils (5×10^4 cells/ml) were cultured at 37° C in humidified air with 5% CO_2 in 25 cm² tissue culture flasks in a total volume of 5 to 10 ml. The number of viable cells on day 7 as determined by trypan blue exclusion was 60% (range, 41% to 89%) compared with that before culturing.

TABLE I. Histamine release from purified basophils (day 0)

Secretagogue	Nonreleasers		Releasers		<i>p</i> Value*
	HR (%)	<i>n</i>	HR (%)	<i>n</i>	
Anti-IgE					
100,000×	0.1 ± 0.1	7	14.9 ± 3.5	8	<0.01
20,000×	0.4 ± 0.2	7	43.5 ± 4.1	8	<0.01
5,000×	1.7 ± 0.4	7	57.0 ± 3.4	8	<0.01
1,000×	2.7 ± 0.7	7	55.6 ± 3.2	8	<0.01
Ionophore					
0.05 μg/ml	1.2 ± 0.8	4	0.5 ± 0.5	5	NS
0.1 μg/ml	8.6 ± 4.9	4	5.4 ± 2.3	5	NS
0.2 μg/ml	41.2 ± 5.0	4	53.8 ± 9.0	5	NS
FMLP					
10 ⁻⁶ mol/L	35.6 ± 2.8	4	54.1 ± 8.8	6	NS
10 ⁻⁵ mol/L	36.5 ± 2.5	4	56.0 ± 8.4	6	NS
10 ⁻⁴ mol/L	37.7 ± 4.4	4	52.6 ± 8.0	6	NS
Histamine (pg/cell)	1.4 ± 0.1	7	1.2 ± 0.1	8	NS

HR, Histamine release; NS, not significant.

*Values reflect differences between releaser and nonreleaser groups.

Amplification of messenger RNA of FcεRIα, β, γ subunits by reverse transcriptase-polymerase chain reaction

Total RNA was isolated from the leukocytes by Trisolb (Biotecx, Houston, Texas) according to the manufacturer's instructions. The first-strand complementary DNA was synthesized from the total RNA by using oligo dT and reverse transcriptase according to a standard method. Briefly, the total RNA (5 μg) with oligo (dT) 12-18 (0.5 mg, Pharmacia) was incubated at 70° C for 10 minutes and quickly chilled on ice. Then 200 U of reverse transcriptase (Superscript, Gibco) with deoxyribonucleoside triphosphate mixture, dithiothreitol, and first-strand buffer was added to the mixture for reverse transcription and incubated at 42° C for 50 minutes and subsequently at 90° C for 5 minutes and chilled on ice according to the manufacturer's instructions. The remaining RNA was degraded by ribonuclease (2.6 U, Gibco) at 37° C for 20 minutes.

The second-strand DNA synthesis, 40-cycle amplification for FcεRIα and β, and 30-cycle amplification for FcεRIγ were performed with a DNA Thermal Cycler (Perkin Elmer, Foster City, Calif.). To the first-strand cDNA, which was used as a template corresponding to 0.5 μg of total RNA, 1 nmol of each direct and reverse primer, deoxyribonucleoside triphosphate mixture, Ampli Taq DNA polymerase (5 U, Perkin Elmer), and polymerase enhancer were added with DNA polymerase buffer; and the mixture was applied to the thermal cycler for amplification. Direct and reverse oligo primers for FcεRI α, β, and γ were constructed to amplify the full coding sequences according to the published sequences. For FcεRIα, the direct primer for PCR is 5'-CATGCTCGAGATGGCGCCTGCCATGGAA-3' (nucleotide number, -10 to 18), and the reverse primer is 5'-CATGGCGGCCGCTCAGT-

TGTTTTTGGGGTTTGG-3' (786 to 754). For FcεRIβ, the direct primer for PCR is 5'-CTGCTCGAGATGGACACAGAAAGTAATAGG-3' (-8 to 21), and the reverse primer is 5'-CATGGCGGCCGCTTATAAATCAATGGGAGGAGACATTTCCCC-3' (747 to 706). For FcεRIγ, the direct primer for PCR is 5'-CTGCTCGAGATGAT-TCCAGCAGTGGTCTTG-3' (-8 to 21), and the reverse primer is 5'-TCTAGCGGCCGCTACTGTGGTGGTT-TCTC-3' (269 to 244).¹⁴⁻¹⁷

Sequencing of the PCR-amplified products

Excess PCR primers were removed from the amplified products by using Suprec-02 (Takara, Kyoto, Japan). Dye-deoxy terminator reaction was performed with these purified samples with a Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Norwalk, Conn.) according to the manufacturer's instructions, and the samples were sequenced in both directions by using an automated DNA sequencer (373A Sequencer, Applied Biosystems). Direct and reverse sequencing primers for sense and antisense chains of FcεRI α, β, and γ were constructed on the basis of published sequences.¹⁴⁻¹⁷

Surface IgE density determination

Basophil surface IgE was measured as described by Nguyen et al.⁵

Statistical analysis

For statistical evaluation of data from nonreleasers and releasers, Student's *t* test was used. Percent histamine release of basophils before and after culturing was compared by means of paired *t* test. Data are expressed as means ± SEM.

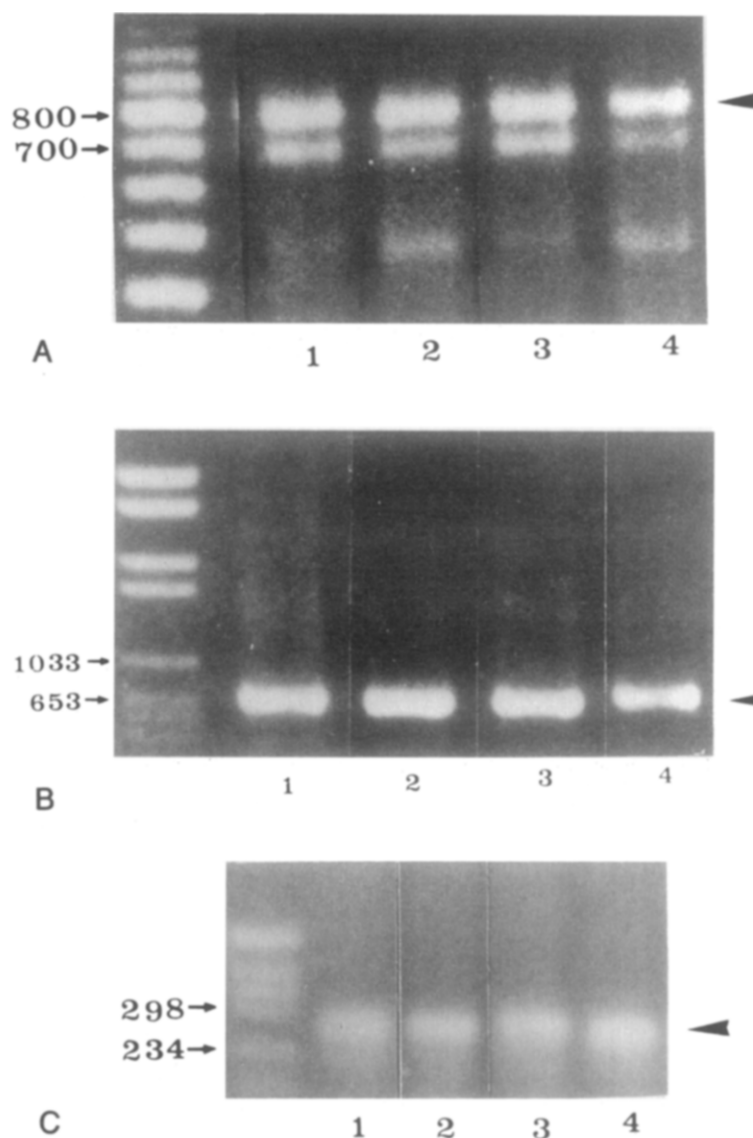


FIG. 1. PCR amplification of cDNA specific for FcεRIα (**A**), β (**B**), and γ (**C**). The amplified cDNAs from leukocytes of nonreleasers (*lanes 1 and 2*) and releasers (*lanes 3 and 4*) were resolved on 1% agarose gel containing ethidium bromide and visualized by exposure to UV rays. The main band for FcεRIα was observed at a size of around 800 bp (deduced size, 796 bp) in both nonreleaser and releaser samples. Another smaller-sized band was sometimes detected in both samples, but sequencing data completely matched the published sequence. The main band for FcεRIβ was observed at a size of around 750 bp (**B**) and for FcεRIγ, 290 bp (**C**) in both samples, and the sequence data of both samples completely matched the ones that have been published.

RESULTS

Basophils were purified from peripheral blood by Percoll density centrifugation, followed by negative panning selections. The donors were divided into releasers (maximal histamine release >5%) and nonreleasers (<5%) according to the response of purified cells to anti-IgE. Comparative studies of purified cells and unfractionated leukocytes indicated that the lack of responsiveness to anti-

IgE did not result from selection of cells through purification procedures (data not shown). The profiles of histamine release in basophils of releasers and nonreleasers are summarized in Table I. Basophils isolated from nonreleasers exhibited a release profile consistent with the report by Nguyen et al.⁵ Despite the lack of IgE-mediated degranulation, anti-IgE nonreleasing basophils released histamine in response to stimuli, such as

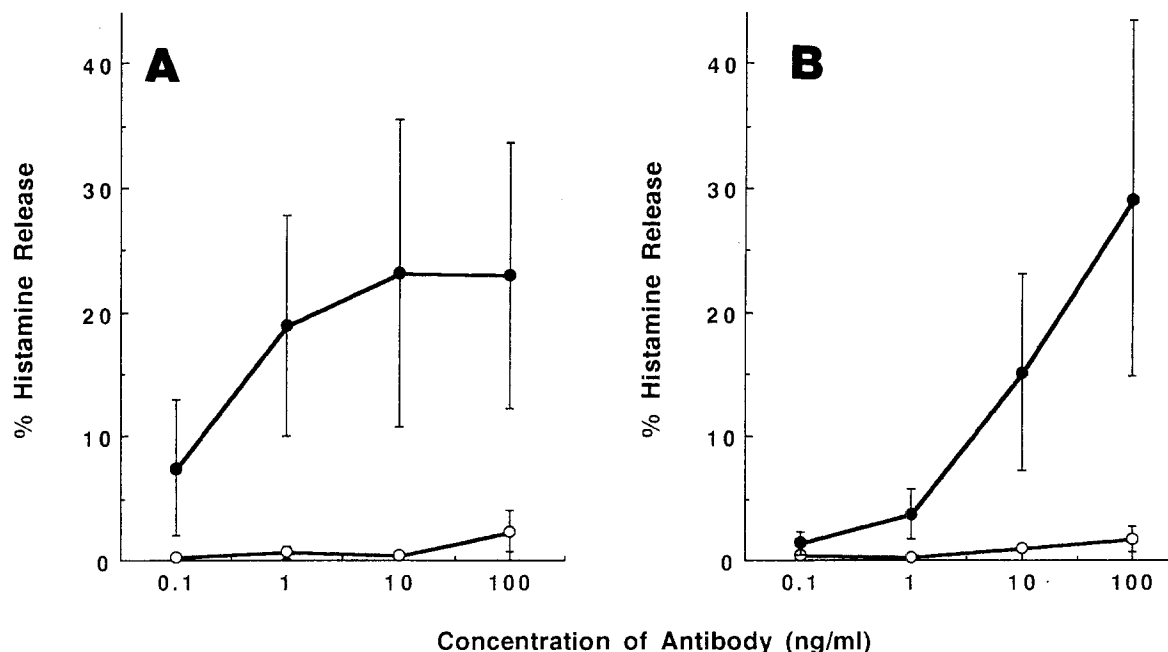


FIG. 2. Basophil histamine release evoked by anti-FcεRI antibodies: releasers versus nonreleasers. Purified basophils of releasers (●, *n* = 3) and nonreleasers (○, *n* = 3) were challenged with serially diluted monoclonal (A) and polyclonal (B) anti-FcεRI antibodies. Bars represent standard error of the mean.

TABLE II. Histamine release from purified basophils (day 7)

Secretagogue	Nonreleasers			Releasers			<i>p</i> Value‡
	HR (%)	<i>n</i>	<i>p</i> Value*	HR (%)	<i>n</i>	<i>p</i> Value†	
Anti-IgE							
100,000×	18.7 ± 5.3	7	<0.01	26.4 ± 7.0	8	NS	NS
20,000×	32.7 ± 5.7	7	<0.01	38.6 ± 7.2	8	NS	NS
5,000×	26.7 ± 4.9	7	<0.01	35.5 ± 5.7	8	<0.01	NS
1,000×	15.7 ± 4.1	7	<0.01	22.0 ± 5.4	8	<0.01	NS
Ionophore							
0.05 μg/ml	0.6 ± 0.6	4	NS	4.1 ± 2.7	5	NS	NS
0.1 μg/ml	1.3 ± 1.3	4	NS	9.7 ± 5.0	5	NS	NS
0.2 μg/ml	11.3 ± 2.4	4	<0.05	26.3 ± 10.3	5	<0.01	NS
FMLP							
10 ⁻⁶ mol/L	24.9 ± 7.0	4	NS	26.7 ± 4.7	6	<0.05	NS
10 ⁻⁵ mol/L	25.0 ± 5.0	4	NS	28.0 ± 4.8	6	<0.01	NS
10 ⁻⁴ mol/L	25.4 ± 5.4	4	<0.05	30.0 ± 5.5	6	<0.05	NS

HR, Histamine release; NS, not significant.

*Values reflect differences between day 1 and day 7 in nonreleasers.

†Values reflect differences between day 1 and day 7 in releasers.

‡Values reflect differences between releaser and nonreleaser groups.

FMLP and ionophore A23187, which bypass FcεRI. These results indicate that the non-IgE-mediated signal transduction pathway leading to degranulation is not impaired in nonreleasing basophils.

In the first attempt to study the mechanism of

the deficit of IgE-mediated degranulation in nonreleasers, we attempted to determine whether any variations are present in the mRNA sequence of FcεRI by using an RT-PCR method. All of the main amplified cDNAs for FcεRIα from leukocytes of both releasers and nonreleasers migrated

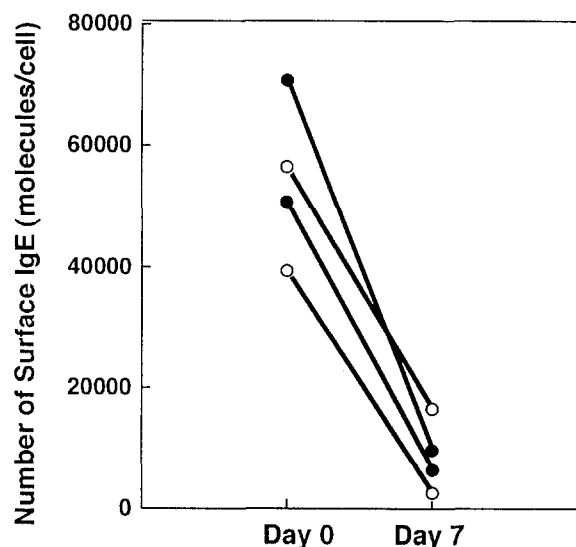


FIG. 3. Number of surface IgE molecules of basophils before and after culturing with IL-3 for 7 days. Purified basophils from releasers (●, $n = 2$) and nonreleasers (○, $n = 2$) were cultured in the presence of 300 pmol/L IL-3 for 7 days. Basophils on day 0 and day 7 were treated with acetate buffer (pH 3.7), and dissociated IgE was determined.

to the position of approximately 800 bp in size (deduced size, 796 bp) on 1% agarose gel, and there was no difference in the size of the main bands, indicating that neither a significant insertion nor deletion occurred in the mRNAs of FcεRIα from these donors. This was also the case with the main amplified cDNAs for FcεRIβ (750 bp) and FcεRIγ (290 bp) (Fig. 1). In addition, the sequence data of cDNA of FcεRIα, β, and γ chains from both nonreleasers' and releasers' basophils completely matched the reported sequences (data not shown). When there were ambiguous assignments for nucleotides in each sequence, we resequenced with other appropriate primers, which should bind near ambiguous parts in both directions, and confirmed the sequence each time. These results indicate that there was no change, at least in the primary structure of FcεRI, in nonreleasing basophils.

Although nonreleasing basophils had an intact primary structure of FcεRI, stimulation of these basophils with antibodies against FcεRI¹⁸ failed to cause histamine release. As shown in Fig. 2, neither mouse monoclonal nor rabbit polyclonal anti-human FcεRI antibodies induced a significant histamine release from nonreleaser basophils, suggesting that the signaling pathway downstream from FcεRI is a locus for the defect

of IgE-mediated degranulation in nonreleasing basophils.

In the next series of experiments, we attempted to determine whether the releasability to anti-IgE of basophils could be changed during long-term culture. Purified basophils were maintained for 7 days in IL-3-containing media, and the degranulation responses to secretagogues were examined in basophils from both releasers and nonreleasers. After 7 days of culture, basophils isolated from all nonreleasers released histamine in response to anti-IgE (Table II). Dose-response curves of anti-IgE in both releaser and nonreleaser basophils were equal; no statistically significant difference was found with any concentration of anti-IgE. It is also noteworthy that long-term culture with IL-3 resulted in an increased sensitivity to anti-IgE in releaser basophils; the optimal concentration of anti-IgE shifted from 1:5000 on day 0 (shown in Table I) to 1:20,000 on day 7 in releasing basophils, and nonreleasing basophils showed a maximal release at the same anti-IgE concentration (1:20,000) on day 7. No statistical significance was observed in basophils from releasers and nonreleasers, which were maintained for 7 days, when histamine release was initiated by FMLP and ionophore A23187. In releasing basophils, however, a significant decrease in histamine release was observed with 1 ($p < 0.05$), 10 ($p < 0.01$), and 100 μmol/L ($p < 0.05$) FMLP.

It has been clearly demonstrated that the number of surface IgE molecules of peripheral basophils is independent of their releasability.^{19,20} We examined the change in the number of surface IgE molecules before and after culturing. As shown in Fig. 3, the numbers of IgE molecules on day 7 declined to approximately one tenth of those on day 0 in both releasers and nonreleasers; there was no apparent difference between releasers and nonreleasers.

We previously reported that IL-3 primes basophils for increased releasability and that the priming effect is a rapid process (within 15 minutes of preincubation).²¹ Therefore in the last series of experiments, we examined the time kinetics for the induction of the IgE-mediated releasability change in nonreleasing basophils. As shown in Fig. 4, nonreleasing basophils did not release histamine in response to anti-IgE after 1 day of culturing. However, after 3 to 7 days, the basophils acquired the potency to release histamine on stimulation with anti-IgE. These results indicate that a long incubation period is necessary for nonreleaser

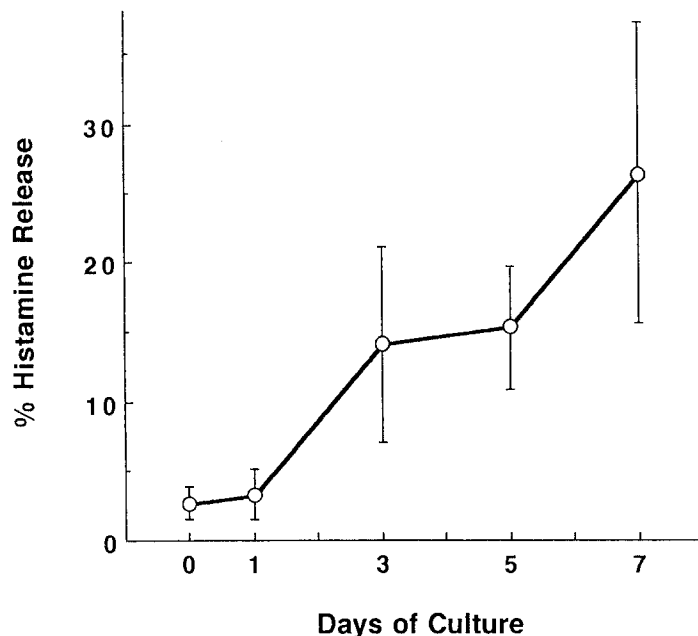


FIG. 4. Anti-IgE-induced histamine release from nonreleasing basophils after various periods of culturing with 300 pmol/L IL-3. Purified basophils from three different nonreleasers were cultured for the indicated periods, and histamine release was evoked by serially diluted anti-IgE. The average percent histamine release at optimal concentrations of anti-IgE is indicated. Bars represent standard error of the mean.

basophils to acquire the responsiveness to anti-IgE stimulation.

DISCUSSION

The presence of basophils that fail to respond to IgE cross-linking stimuli was first recognized more than 20 years ago⁶; however, it is only in recent years that the control mechanism regulating releasability has become an area of active investigation. In this study, experiments with RT-PCR showed no mutation of FcεRI in nonreleasing basophils; the sequence data of cDNAs of FcεRIα, β, and γ from nonreleaser basophils indicated that there is no change, at least in the primary structure of their FcεRI, compared with that of releaser basophils (Fig. 1). However, despite the intact structure of FcεRI, stimulation with antibodies against FcεRI failed to induce degranulation in nonreleasing basophils (Fig. 2). These findings indicate that the unresponsiveness to IgE-mediated stimulation does not result from an insufficient cross-linking of surface IgE and that the defect in the FcεRI-mediated signal transduction pathway must be located downstream of the receptor itself.

IL-3 has been shown to exhibit diverse effects on human basophils. It acts on basophil progenitors to

induce proliferation and differentiation.²²⁻²⁴ In addition to its effect on immature progenitors of basophil lineage, IL-3 also acts on their mature progeny. We and others have shown that IL-3 primes peripheral basophils for increased mediator release^{21, 25} and exerts a migratory effect on basophils.¹¹ We have also demonstrated that IL-3 prolongs the *in vitro* life span of basophils,¹⁰ possibly by rescuing them from programmed cell death. In this study we demonstrated that culturing of nonreleasing basophils in IL-3-containing media resulted in the acquisition of responsiveness to IgE-mediated stimulation (Tables I and II). Inasmuch as basophils deceased so rapidly in the absence of IL-3, we could not determine whether the conversion in fact could occur in nonreleasing basophils cultured without IL-3. However, no relationship between releasability after culturing with IL-3 and basophil purities was observed when different basophil preparations with different purities were used for culturing (data not shown), suggesting that the effect of IL-3 is not mediated indirectly through contaminating cells, but rather that it is mediated directly through interaction with basophils.

Conversion of nonreleasing basophils required long-term culturing with IL-3 (>3 days, Fig. 4).

These results are in marked contrast to our previous report demonstrating that the priming effect of IL-3 was completed with short-term preincubation (< 15 minutes).²¹ Long-term incubations with IL-3 might induce qualitative change in basophils. Although tyrosine phosphorylation is perceived to play a role in the acute priming effect of IL-3,²⁶ the requirement of long-term culture indicates that different mechanisms, such as *de novo* protein synthesis, are involved in the conversion of nonreleasing basophils.

Cross-linking of FcεRI has been shown to transduce intracellular signals through diacylglycerol and 1, 4, 5-inositol trisphosphate as second messengers.^{27, 28} Inositol trisphosphate induces the elevation of cytosolic calcium, and diacylglycerol activates protein kinase C, which subsequently leads to exocytosis of basophilic granules. A previous report has demonstrated that nonreleasing basophils release histamine in response to ionophore and 12-O-tetradecanoylphorbol 13-acetate, which bypass the steps preceding the elevation of cytosolic calcium or the activation of protein kinase C, respectively.⁵ Thus from these results, it can be postulated that nonreleasing basophils lack early components of the signal transduction pathway (i.e., generation of second messengers downstream of FcεRI). Warner and MacGlashan²⁹ demonstrated that overall IgE-mediated histamine release correlates well with the magnitude of the calcium response in releasers, as well as nonreleasers. Knol et al.⁷ also reported that nonreleasing basophils fail to induce the Ca²⁺ influx after stimulation with anti-IgE. The rise of cytosolic calcium might be an essential component that determines releasability, and the long-term culturing with IL-3 might convert nonreleasing basophils to releasing basophils by enhancing the machinery for the elevation of cytosolic calcium. Support for this hypothesis can be found in reports by MacGlashan et al.,^{30, 31} who demonstrated that long-term incubations with IL-3 caused a marked increase in the basophil cytosolic free calcium response, which follows stimulation, although short-term pretreatments with IL-3 have very little effect on the calcium response. Analysis of the cytosolic calcium change in nonreleasing basophils maintained with IL-3 would be helpful to clarify this point.

Several lines of evidence reveal that nonreceptor tyrosine kinases, Lyn and Syk, which associate with β and γ chains, respectively, play crucial roles in intracellular calcium elevation.^{32, 33} The intact primary structure of FcεRI of nonreleaser basophils

indicates that downstream signaling molecules, including Lyn and Syk, may be altered. We are now exploring the expression and function of these molecules.

In summary, we demonstrated that nonreleasing basophils converted to releasing basophils by long-term culturing with IL-3. By extension of this experiment to encompass the biochemical identification of the precise steps recovered during long-term culturing with IL-3, we will be able to explain the mechanism causing the lack of responsiveness in the anti-IgE nonreleasing basophils.

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