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Immunology

Two forms of the low-affinity Fc receptor for IgE differentially mediate endocytosis and phagocytosis: Identification of the critical cytoplasmic domains

(CD23/endocytosis/phagocytosis)

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ABSTRACT **We have previously identified two species of the low-affinity human Fc receptor for IgE, FcεRIIa and FcεRIIb, which differ only in a short stretch of amino acids at the N-terminal cytoplasmic end. Their differential expressions on B cells and monocytes suggest that FcεRIIa and FcεRIIb are involved in B-cell function and IgE-mediated immunity, respectively. Here we show that FcεRII-mediated endocytosis is observed only in FcεRIIa-expressing cells, whereas IgE-dependent phagocytosis is observed only in FcεRIIb-expressing cells, demonstrating the functional difference between FcεRIIa and FcεRIIb. Furthermore, site-directed mutagenesis revealed that the tyrosine residue in the FcεRIIa-specific region is important for endocytosis, and the Asn-Pro residues in the FcεRIIb-specific region are required for phagocytosis. These findings suggest that endocytosis and phagocytosis are functionally separable phenomena involving distinct amino acid residues.**

Subpopulations of leukocytes express the low-affinity Fc receptor for IgE (FcεRII), which is believed to have multiple functions in B lymphocytes and in effector cells of IgE-mediated immunity (1–3). FcεRII has been known as a B-cell differentiation antigen, CD23 (4–7), and has been proposed to be involved in several B-cell functions such as the growth-promoting effect (8, 9), cell adhesion (10), and IgE-mediated antigen presentation (11). On the other hand, FcεRII has also been reported to be expressed on macrophages, eosinophils, and platelets and to be an effector molecule of IgE-mediated immunity, including immediate-type allergy and cytotoxicity against certain parasites (2). IgE can mediate release of varieties of chemical mediators from eosinophils, monocytes, and platelets (12–14).

We have previously identified two species of human FcεRII, FcεRIIa and FcεRIIb (15, 16). They are generated by utilizing different transcriptional initiation sites and 5′ exons of the single genomic gene, and they differed only in a short amino acid stretch in the N-terminal cytoplasmic end. FcεRIIa is constitutively, but cell-type-specifically, expressed on B cells. On the other hand, monocytes and eosinophils express only FcεRIIb. FcεRIIb is expressed on normal peripheral blood B cells and monocytes only after stimulation with interleukin 4, which is known to be the cytokine responsible for the isotype switching of B cells to IgE (17–19). Moreover, peripheral blood lymphocytes from atopic individuals expressed FcεRIIb without stimulation with interleukin 4 (16). The differential manner of gene expression for these receptors suggests that FcεRIIa and FcεRIIb may be involved in B-cell function and IgE-mediated immunity, respectively.

In this paper, we have attempted to elucidate the molecular basis of the functional difference between these two forms of receptor molecules by using stable transfectants expressing either human wild-type or mutated FcεRII and have demonstrated that endocytosis is mediated only through FcεRIIa and that phagocytosis is mediated only through FcεRIIb. Furthermore, the minimum amino acid residues necessary for endocytosis and phagocytosis have been determined. The results indicate that endocytosis and phagocytosis of FcεRII are independent phenomena mediated through distinct amino acid residues.

MATERIALS AND METHODS

Establishment of Wild-Type and Mutant FcεRII-Expressing Transfectants. The FcεRIIb cDNA (16) was cloned into the M13 phage vector. Each of the mutations was introduced into the region of FcεRIIb encoding the cytoplasmic domain by using the Amersham *in vitro* mutagenesis kit according to the manufacturer's instruction. The FcεRIIa (15), FcεRIIb, and mutant FcεRIIb cDNAs were cloned into the pZip-neo vector and transfected into ψ2 cells by calcium phosphate coprecipitation. After G418 selection, cells that strongly expressed FcεRII epitopes were selected by using a fluorescence-activated cell sorter (FACS). J774 macrophage cells were infected with retroviruses carrying wild-type or mutant FcεRII sequences by coculturing with the above ψ2 transfectants. J774 cells strongly expressing FcεRII determinants were sorted by a FACS.

Endocytosis Assay. Cells were preincubated with ^{125}I-labeled 3-5 anti-FcεRII monoclonal antibody at 4°C for 30 min, washed, and incubated at 37°C in RPMI 1640 medium supplemented with 20 mM Hepes (pH 7.4) and bovine serum albumin (5 mg/ml) for the time indicated. Then, surface-bound ^{125}I-labeled 3-5 antibodies were acid-stripped with citrate buffer [10 mM sodium citrate/0.14 M NaCl/bovine serum albumin (50 μg/ml), pH 2.0], and acid-inaccessible internalized antibodies were measured by a gamma counter.

Phagocytosis Assay. Cells were preincubated with or without interferon γ (IFN-γ) at 200 units/ml for 30 h and were incubated with human IgE conjugated to sheep erythrocytes (SRBCs) by using a chromium chloride method. After a 60-min incubation at 37°C, cells were washed with phosphate-buffered saline and then with 0.16 M NH_4Cl to lyse extra-

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Abbreviations: FcεRII, low-affinity Fc receptor for IgE; SRBC, sheep erythrocyte; FACS, fluorescence-activated cell sorter; IFN-γ, interferon γ.

A

	1	2	3	4	5	6	7	8	9	10
FcεRIIa	Met -	Glu -	Glu -	Gly -	Gln -	Tyr -	Ser	Glu -	Ile -	Glu
FcεRII b		Met -	Asn -	Pro -	Pro -	Ser -	Gln	Glu -	Ile -	Glu
		1	2	3	4	5	6	7	8	9

B

Mutant	Sequence
b-Phe5	Met - Asn - Pro - Pro - Phe - Gln - Glu - Ile - Glu
b-Arg4	Met - Asn - Pro - Arg - Ser - Gln - Glu - Ile - Glu
b-Arg3	Met - Asn - Arg - Pro - Ser - Gln - Glu - Ile - Glu
b-Lys2	Met - Lys - Pro - Pro - Ser - Gln - Glu - Ile - Glu
b-Δ2-6	Met - Glu - Ile - Glu

FIG. 1. Amino acid sequences of wild-type FcεRIIa and FcεRIIb and FcεRIIb mutants. (*A*) The amino acid sequences of N-terminal cytoplasmic ends of FcεRIIa and FcεRIIb. Sequences downstream of the vertical line are identical. The 5-amino acid pattern reported by Vega and Strominger (20) is found in FcεRIIa and is double-underlined (see *Discussion*). The incomplete 5-amino acid pattern found in FcεRIIb is underlined. (*B*) The N-terminal sequences of mutant FcεRIIb. The receptors named b-Phe5, b-Arg4, b-Arg3, b-Lys2, and b-Δ2-6 were mutated, respectively, so that Ser-5 was changed to Phe, Pro-4 was changed to Arg, Pro-3 was changed to Arg, Asn-2 was changed to Lys, and amino acids from positions 2 to 6 were deleted.

cellular SRBCs. Cells were then fixed with 100% ethanol, stained with hematoxylin/eosin, and photographed.

Immunoelectron Microscopic Observation. Cells were incubated with the 3-5 antibody at 4°C for 30 min and at 37°C for 2 min, as described in the endocytosis assay, and fixed for 10 min with 2% (wt/vol) paraformaldehyde/0.1% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). After incubation with rabbit anti-mouse IgG (0.1 mg/ml) for 60 min and then with 4-nm protein A–gold complex (OD_{525} = 0.020) for 30 min, cells were fixed in 2% glutaraldehyde and then in 1% OsO_4 and embedded in Epon.

RESULTS

FcεRIIa but Not FcεRIIb Mediates Efficient Endocytosis in Transfected Cells. FcεRIIa and FcεRIIb differ only in several amino acid residues at the N-terminal cytoplasmic domain as shown in Fig. 1*A*. If these receptors are also functionally different, these regions must contain critical sequences. We first examined the ability of these two forms of FcεRII to mediate endocytosis. A mouse fibroblast cell line ψ2 was transfected with human FcεRIIa or FcεRIIb cDNA. Resulting transfectants were found to express comparable amounts of FcεRII (Fig. 2*A*). They were incubated with ^{125}I-labeled 3-5 anti-FcεRII monoclonal antibody (3) and examined for their ability to internalize labeled antibody. As shown in Fig. 2*B*, the amount of internalized antibody (acid inaccessible) rose rapidly and reached a plateau within 60 min in cells expressing FcεRIIa, whereas the internalization was severely impaired in cells expressing FcεRIIb. The same results were obtained in a murine myeloid leukemia cell line M1 and a murine macrophage cell line J774 transfected with either human FcεRIIa or FcεRIIb cDNA (data not shown). These findings show that FcεRIIa but not FcεRIIb mediates efficient endocytosis.

Restoration of Endocytosis in the Mutant FcεRIIb with an Amino Acid Substitution in the N-Terminal Cytoplasmic Domain. It has been suggested that aromatic amino acids in the cytoplasmic region are required for efficient endocytosis of various receptors (21–23). FcεRIIa contains a tyrosine residue at position 6 whereas FcεRIIb has a serine residue at its equivalent position as shown in Fig. 1*A*. Thus, we substituted phenylalanine for serine at position 5 of FcεRIIb (Fig. 1*B*). This mutant receptor was also expressed on ψ2 cells (Fig. 2*C*). As shown in Fig. 2*D*, the ability of this mutant receptor to mediate endocytosis was significantly restored. Therefore, impaired endocytosis of FcεRIIb can be explained by a lack of aromatic amino acid at position 5 in the cytoplasmic domain.

The distribution of FcεRII on transfectants was also examined by immunogold electron microscopy. As shown in Fig. 3, gold particles were found in more than two-thirds of the coated pits of cells expressing FcεRIIa, whereas most of coated pits did not contain gold particles in FcεRIIb-expressing cells. Furthermore, >60% of the total coated pits of cells expressing the mutant FcεRIIb (b-Phe5) contained immunogold-labeled receptors as expected. The average number of gold particles in a coated pit in cells expressing FcεRIIa, FcεRIIb, and b-Phe5 was 2.09, 0.17, and 1.55, respectively. These results suggest the importance of aro-

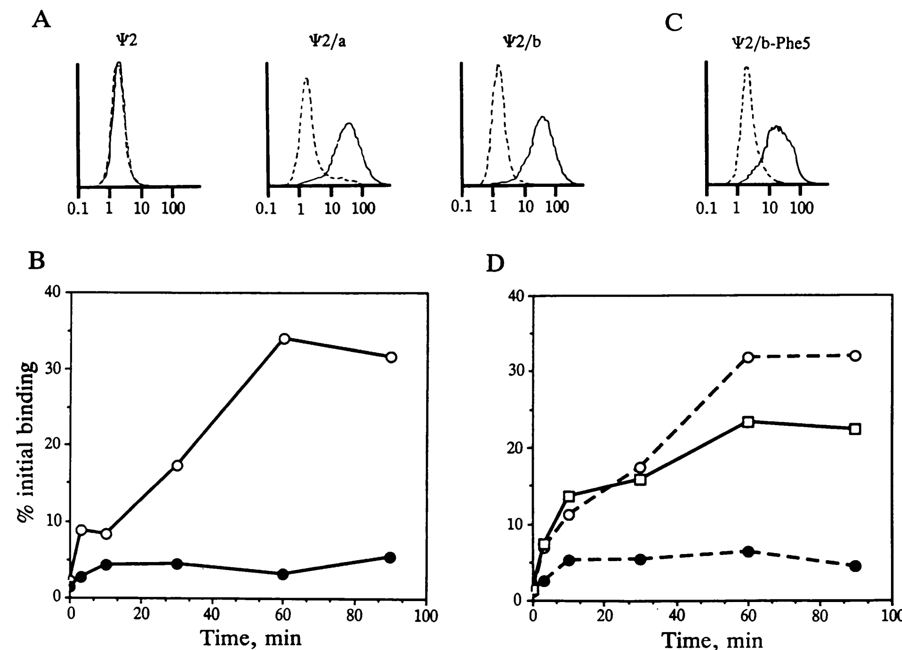

FIG. 2. FACS analysis and endocytosis assay of stable ψ2 transfectants expressing wild-type FcεRIIa and FcεRIIb and mutant b-Phe5. In *A* and *C*, the indicated transfectants were stained with biotinylated 8-30 anti-FcεRII antibody and fluorescein isothiocyanate-conjugated avidin and analyzed by FACS. ψ2/a, ψ2/b, and ψ2/b-Phe5 are ψ2 transfectants expressing FcεRIIa, FcεRIIb, and b-Phe5, respectively. Dotted lines show background autofluorescence levels of each transfectant. The *x* and *y* axes represent the logarithm of fluorescence intensity and the relative cell number, respectively. In *B* and *D*, the ability of each transfectant (○, ψ2/a; ●, ψ2/b; □, ψ2/b-Phe5) to internalize ^{125}I-labeled 3-5 anti-FcεRII antibody was assessed by the amount of acid-inaccessible (endocytosed) radioactivity at time indicated. Values are expressed as a mean percent of the total cell-associated radioactivity at time 0 in three experiments. This 3-5 monoclonal antibody (IgG1) did not bind to the parent ψ2 line (data not shown).

matic amino acids in certain positions in targeting of FcεRII to coated pits.

IgE-Dependent Phagocytosis Was Observed in FcεRIIb-Expressing Transfectants but Not FcεRIIa-Expressing Transfectants. We tested the ability of FcεRIIa and FcεRIIb to mediate IgE-dependent phagocytosis, since FcεRIIb is expressed on phagocytes such as macrophages and eosinophils. Murine macrophage J774 lines expressing either human FcεRIIa or FcεRIIb were established (Fig. 4*A*). A J774 line, which expresses FcγRII, has been shown to mediate IgG-dependent phagocytosis after stimulation with IFN-γ (24). We therefore examined the ability of these J774 transfectants expressing either FcεRIIa or FcεRIIb to engulf IgE-opsonized SRBCs after stimulation with IFN-γ. As shown in Fig. 4*B*, internalized SRBCs were seen in FcεRIIb-expressing J774 cells but not in FcεRIIa-expressing cells. In the absence of IFN-γ stimulation, no phagocytosis was observed in either transfectant. Furthermore, both of the transfectants could engulf comparable numbers of IgG-opsonized SRBCs when stimulated with IFN-γ (data not shown), indicating that they retained FcγR-mediated phagocytic activity. These findings show that FcεRIIb but not FcεRIIa is responsible for IgE-dependent phagocytosis and support the hypothesis that FcεRIIb functions as an effector molecule in IgE-mediated immunity.

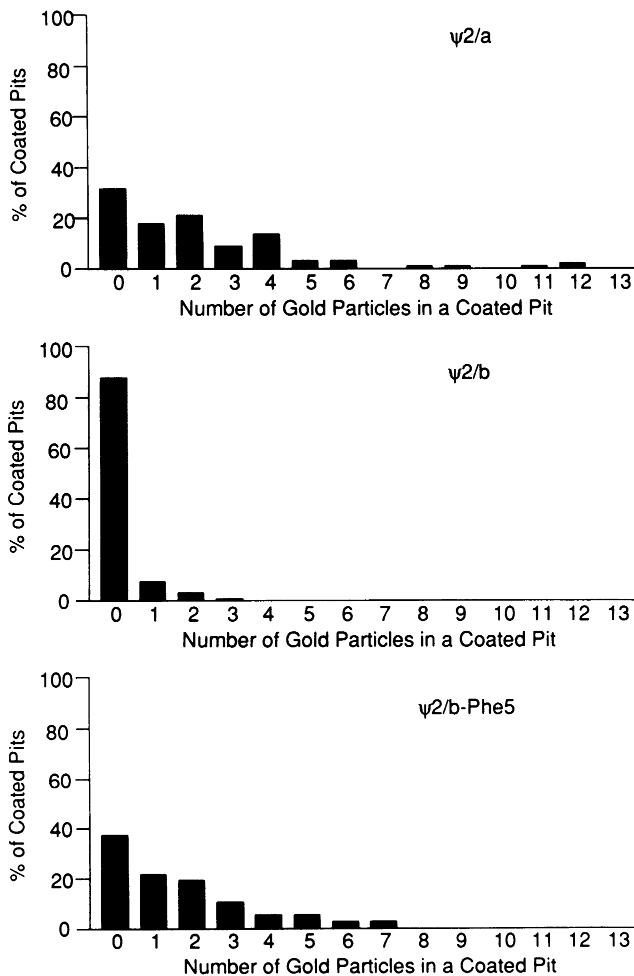

FIG. 3. Immunogold localization of wild-type and mutant FcεRIIs on transfectants. Cells were incubated with anti-FcεRII antibody 3-5 for 15 min at 4°C and for 2 min at 37°C and then fixed. After fixation, cells were incubated with rabbit anti-mouse IgG and 4-nm protein A–gold complexes and then processed for electron microscopy. ψ2/a, ψ2/b, and ψ2/b-Phe5 are ψ2 transfectants expressing FcεRIIa, FcεRIIb, and b-Phe5, respectively. The number of gold particles in coated pits of each transfectant was counted.

Asparagine and Proline Residues in the FcεRIIb-Specific Amino Acid Sequence Are Required for IgE-Dependent Phagocytosis. We then attempted to identify the minimum structure required for phagocytosis. FcεRIIb cDNAs with mutations in the FcεRIIb-specific cytoplasmic region were constructed by site-directed mutagenesis (Fig. 1*B*) and were expressed in a J774 murine macrophage line (Fig. 5*A*). Their ability to mediate phagocytosis of human IgE-opsonized SRBCs was examined after stimulation with IFN-γ. As shown in Fig. 5*B*, the deletion of all the FcεRIIb-specific amino acid residues (b-Δ2-6 in Fig. 1*B*) resulted in the abrogation of phagocytosis. An amino acid substitution at position 4 or 5 of FcεRIIb (b-Arg4 and b-Phe5 in Fig. 1*B*) did not affect the phagocytic activity. However, the phagocytic ability was severely impaired by alteration of an amino acid residue at position 2 or 3 of FcεRIIb (b-Lys2 and b-Arg3 in Fig. 1*B*). These findings clearly show that two amino acid residues, asparagine and proline, at positions 2 and 3 of FcεRIIb are essential for phagocytosis.

DISCUSSION

FcεRIIa but not FcεRIIb mediated endocytosis. A site-directed mutagenesis experiment demonstrated the importance of an aromatic amino acid residue in endocytosis of FcεRII and particularly in targeting FcεRIIa to coated pits. These results are in good agreement with reports on the low density lipoprotein receptor (21), the mannose-6-phosphate receptor (23), the influenza virus hemagglutinin (22), and the transferrin receptor (25), showing that an aromatic amino acid is particularly critical for endocytosis by coated pits. Vega and Strominger (20) reported that some cell surface molecules known to be internalized in coated pits share a common structural feature in a specific cytoplasmic domain composed of five amino acid residues, in which the first residue is aromatic and the others are classified according to polarity and charge. This 5-amino acid sequence is also found in the cytoplasmic domain of FcεRIIa beginning at Tyr-6 (Tyr-Ser-Glu-Ile-Glu). The Ser-5 → Phe substitution in FcεRIIb resulted in formation of the proposed pentameric consensus, restoration of endocytosis, and targeting this receptor to coated pits. Murine FcεRII, which is equivalent to human FcεRIIa and also has a tyrosine residue at the same position (26), has been demonstrated to efficiently focus and present antigens to T cells through IgE antibodies (11), suggesting that the murine counterpart has endocytosis activity as well. However, murine FcεRII does not display the pentameric consensus but does contain two more aromatic residues, tyrosine and tryptophan (Tyr-Ser-Gly-Tyr-Trp). Thus, the aromatic nature of certain amino acids rather than the proposed pentameric sequence might be important in endocytosis. Recently, internalization sequences of low density lipoprotein receptor and transferrin receptor have been shown to be Asn-Pro-Xaa-Tyr and Tyr-Xaa-Arg-Phe, respectively (26, 27). The sequence around Tyr-6 of FcεRIIa is dissimilar to both of them. Collawan *et al.* (27) have suggested that both Tyr-Xaa-Arg-Phe and Asn-Pro-Xaa-Tyr favor a tight-turn conformation that may be a recognition structure for internalization. It would be interesting to know whether the region around Tyr-6 in FcεRIIa that forms a tight turn or the other unique structure in this receptor may be responsible for endocytosis.

Several receptors including Fc receptors for IgG (FcγRs) and complement receptors mediate phagocytosis. Endocytosis and phagocytosis share a common functional feature of internalizing exogenous molecules through specific surface determinants. Although several differences have been proposed to distinguish between endocytosis and phagocytosis, including the size of internalized materials and an energy dependency (28–30), little is known about the structural

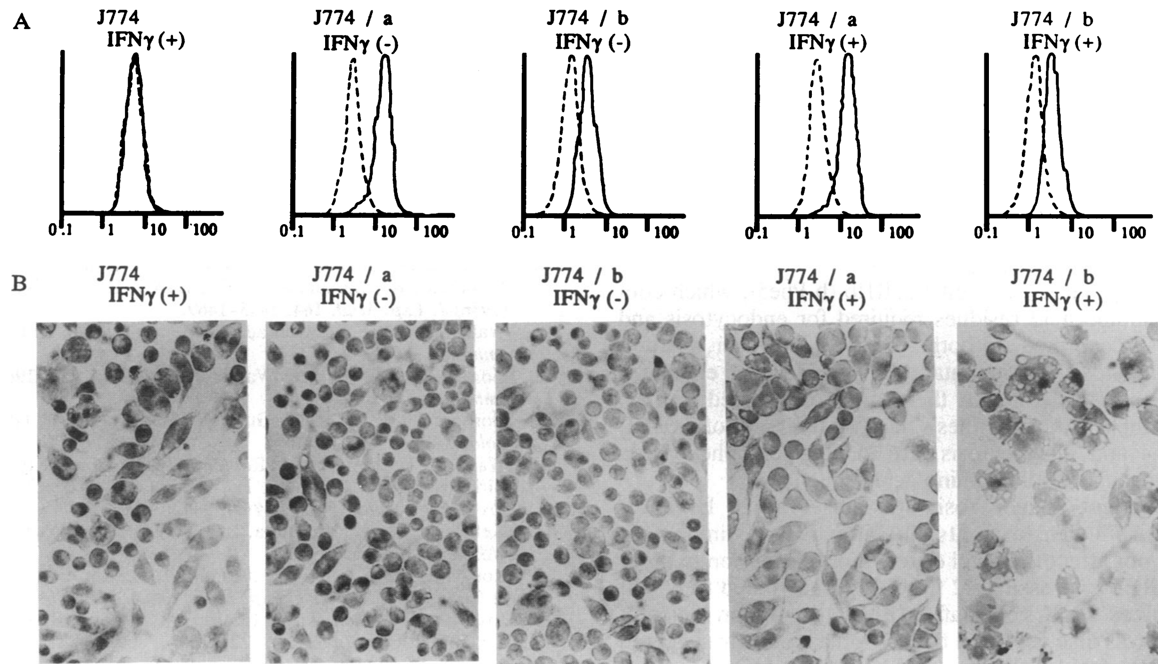

FIG. 4. FACS analysis and IgE-dependent phagocytosis assay of stable J774 transfectants expressing either wild-type FcεRIIa or FcεRIIb stimulated with or without IFN-γ. (*A*) The reactivity of transfectants to anti-FcεRII antibody. The indicated J774 transfectants were stained with biotinylated 8-30 anti-FcεRII antibody and fluorescein isothiocyanate-conjugated avidin and analyzed by using a FACS. J774/a and J774/b are J774 transfectants expressing FcεRIIa and FcεRIIb, respectively. Dotted lines show background autofluorescence levels of each transfectant. The *x* and *y* axes represent the logarithm of fluorescence intensity and the relative cell number, respectively. (*B*) The phagocytosis assay of transfectants. The ability of each transfectant stimulated with or without IFN-γ to engulf IgE-opsonized SRBCs was judged by the existence of SRBCs in the cytoplasm of each transfectant. Engulfed SRBCs are seen only in the cytoplasm of IFN-γ-stimulated J774/b cells. The ability of phagocytosis was also assessed by the number of engulfed SRBCs per 100 cells, which was 8 cells in J774,IFN-γ^+, 6 cells in J774/a,IFN-γ^-, 12 cells in J774/b,IFN-γ^-, 18 cells in J774/a,IFN-γ^+, and 450 cells in J774/b,IFN-γ^+.

requirement for phagocytosis. IgE-dependent phagocytosis was observed only in FcεRIIb-expressing J774 transfectants but not in FcεRIIa-expressing transfectants. This indicates that endocytosis and phagocytosis are completely dissociated in two forms of FcεRII. Furthermore, we showed the requirement of asparagine and proline residues at amino acid

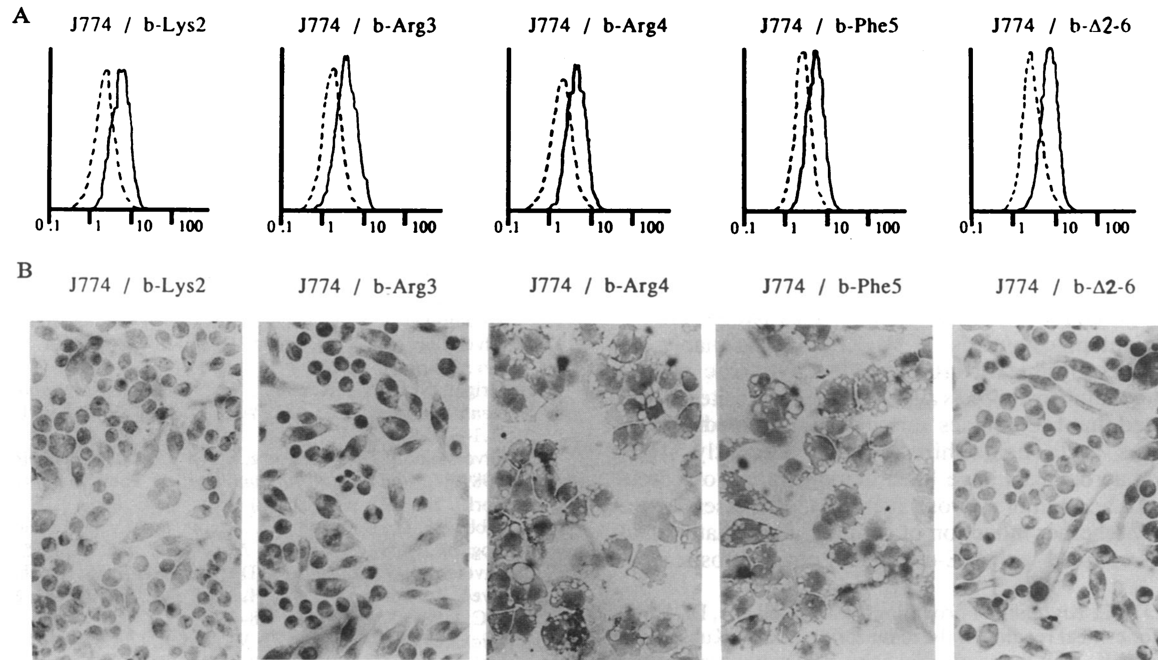

FIG. 5. FACS analysis and IgE-dependent phagocytosis assay of stable J774 transfectants expressing mutant FcεRIIb. (*A*) The expression of mutant FcεRIIb in J774 transfectants. The transfectants expressing b-Lys2, b-Arg3, b-Arg4, b-Phe5, and b-Δ2-6 (from left to right) were stained by the 8-30 antibody and analyzed by FACS after IFN-γ stimulation. Axes are as in Fig. 4. (*B*) The phagocytosis activity of mutant FcεRIIb. Engulfed SRBCs are seen in the cytoplasm of J774/b-Arg4 and J774/b-Phe5 cells stimulated with IFN-γ but not in other cells. The phagocytosis of each transfectant stimulated with IFN-γ to phagocytose SRBCs was also assessed by the number of engulfed SRBCs per 100 cells, which was 15 cells in J774/b-Lys2, 10 cells in J774/b-Arg3, 421 cells in J774/b-Arg4, 430 cells in J774/b-Phe5, and 15 cells in J774/b-Δ2-6.

positions 2 and 3 of FcεRIIb for IgE-dependent phagocytosis. This amino acid sequence was also found within the cytoplasmic domains of FcγRIIs (human FcγRII, murine FcγRIIβ1, and murine FcγRIIβ2) (31, 32) and complement receptor 1 (33). A variant line of J774, which expresses FcγRIIα but not FcγRIIβ, has been reported to engulf IgG-coated SRBCs efficiently. However, FcγRIIα does not display the Asn-Pro sequence. At present, we do not know whether the requirement of this sequence can be generalized for other phagocytic receptors.

It is notable that the mutant FcεRIIb (b-Phe5), which contains both amino acid residues required for endocytosis and phagocytosis, can mediate both activities. However, restoration of endocytosis in this mutant did not affect the efficiency of phagocytosis as defined by the number of engulfed SRBCs. This also supports the hypothesis that two functions of FcεRII, endocytosis and phagocytosis, are independent phenomena mediated through distinct amino acid sequences.

IgE-dependent phagocytosis was observed in FcεRIIb-expressing J774 transformants only after IFN-γ stimulation. IFN-γ did not affect the level of receptor expression in these cells. FcεRIIb-expressing ψ2 fibroblast cells failed to engulf IgE-opsonized SRBCs even after IFN-γ stimulation (data not shown). It was also reported that fibroblast cells transfected with FcγRII cDNA bound but did not engulf IgG-opsonized SRBCs (24). These observations suggest that cellular factor(s) and machinery are required to promote phagocytosis. The existence of associate protein(s) that bind to Asn-Pro residues in the cytoplasmic end of FcεRIIb might explain why IgE-dependent phagocytosis takes place only in a cell-type specific manner and is induced by IFN-γ.

On the basis of their expression pattern, we have proposed (16) that FcεRIIa and FcεRIIb may play distinct roles in B lymphocytes and effector cells of IgE-mediated immunity, respectively. Kehry and Yamashita (11) have demonstrated that murine B cells treated with an IgE monoclonal antibody to trinitrophenyl (TNP) were 100-fold more effective than were untreated B cells in presenting low concentrations of TNP-coupled antigens to T cells and that such high-efficiency IgE-dependent antigen focusing and presentation is mediated by FcεRII. Although the same findings must be reproduced in the human system, an efficient endocytosis activity of FcεRIIa is also likely to contribute to IgE-dependent antigen presentation on human B cells. On the other hand, activated macrophages, which are also efficient antigen-presenting cells, express only FcεRIIb. Therefore, it would be interesting to determine whether IgE-dependent antigen presentation is restricted to particulate antigens in macrophages. Macrophage transfectants described here should provide an experimental system to elucidate the difference in antigen presentation through endocytosis and phagocytosis pathways.

The efficient phagocytosis of FcεRIIb is reasonably assumed to be responsible for IgE-dependent cytotoxic activity of macrophage and eosinophils against certain parasites. It has been reported that IgE induces various chemical mediators in macrophages and eosinophils (12–14). Recently, IgE-stimulated macrophages have been demonstrated to secrete interleukin 1α and tumor necrosis factor α (34). Presently, we do not know whether induction of some or all mediators and cytokines is coupled with IgE-dependent phagocytosis.

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1. Spiegelberg, H. L. (1984) *Adv. Immunol.* **35**, 61–88.
2. Capron, A., Dessaint, J., Capron, M., Joseph, M., Ameisen, J. C. & Tonnel, A. B. (1986) *Immunol. Today* **7**, 15–18.
3. Suemura, M., Kikutani, H., Barsumian, E. L., Hattori, Y., Kishimoto, S., Sato, R., Maeda, A., Nakamura, H., Owaki, H., Hardy, R. R. & Kishimoto, T. (1986) *J. Immunol.* **137**, 1214–1220.
4. Yukawa, K., Kikutani, H., Owaki, H., Yamasaki, K., Yokota, A., Nakamura, H., Barsumian, E. L., Hardy, R. R., Suemura, M. & Kishimoto, T. (1987) *J. Immunol.* **138**, 2576–2580.
5. Bonnefoy, J.-Y., Aubry, J.-P., Peronne, C., Wijdenes, J. & Banchereau, J. (1987) *J. Immunol.* **138**, 2970–2978.
6. Kikutani, H., Suemura, M., Owaki, H., Nakamura, H., Sato, R., Yamasaki, K., Barsumian, E. L., Hardy, R. R. & Kishimoto, T. (1986) *J. Exp. Med.* **164**, 1455–1469.
7. Waldschmidt, T. J., Conrad, D. H. & Lynch, R. G. (1988) *J. Immunol.* **140**, 2148–2154.
8. Gordon, J., Rowe, M., Walker, L. & Guy, G. (1987) *Eur. J. Immunol.* **16**, 1075–1086.
9. Gordon, J., Webb, A. J., Guy, G. R. & Walker, L. (1987) *Immunology* **60**, 517–521.
10. Wang, F., Gregory, C. D., Rowe, M., Rickinson, A. B., Wang, D., Birkenbach, M., Kukutani, H., Kishimoto, T. & Kieff, E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3452–3456.
11. Kehry, M. R. & Yamashita, L. C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7556–7560.
12. Rouzer, C. A., Scott, W. A., Hamill, A. L., Liu, F.-T., Katz, D. H. & Cohn, Z. A. (1982) *J. Exp. Med.* **156**, 1077–1086.
13. Joseph, M., Tonnel, A. B., Capron, A., Arnoux, B. & Benveniste, J. (1983) *J. Clin. Invest.* **71**, 221–230.
14. Khalife, J., Capron, M., Grzych, J. M., Bazin, H. & Capron, A. (1985) *J. Immunol.* **134**, 1968–1974.
15. Kikutani, H., Inui, S., Sato, R., Barsumian, E. L., Owaki, H., Yamasaki, K., Kaisho, T., Uchibayashi, N., Hardy, R. R., Hirano, T., Tsunasawa, S., Sakiyama, F., Suemura, M. & Kishimoto, T. (1986) *Cell* **47**, 657–665.
16. Yokota, A., Kikutani, H., Tanaka, T., Sato, R., Barsumian, E. L., Suemura, M. & Kishimoto, T. (1988) *Cell* **55**, 611–618.
17. Coffman, R. L., Ohara, J., Bond, M. N., Carty, J., Zlotnik, A. & Paul, W. E. (1986) *J. Immunol.* **136**, 4538–4541.
18. Lee, F., Yokota, T., Otsuka, T., Meyerson, P., Villaret, D., Coffman, R., Mosman, T., Rennick, D., Roehm, N., Smith, C., Zlotnik, A. & Arai, K. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2061–2064.
19. Snapper, C. M. & Paul, W. E. (1987) *Science* **236**, 944–947.
20. Vega, M. A. & Strominger, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2688–2692.
21. Davis, C. G., van Driel, I. R., Russell, D. W., Anderson, R. G. W., Brown, M. S. & Goldstein, J. L. (1987) *J. Biol. Chem.* **262**, 4075–4082.
22. Lazarovits, J. & Roth, M. (1988) *Cell* **53**, 743–752.
23. Lobel, P., Fujimoto, K., Ye, R. D., Griffithes, G. & Kornfeld, S. (1989) *Cell* **57**, 787–796.
24. Weinshank, R. L., Luster, A. D. & Ravetch, J. V. (1988) *J. Exp. Med.* **167**, 1909–1925.
25. Jing, S., Spencer, T., Miller, K., Hokins, C. & Trowbridge, I. S. (1990) *J. Cell Biol.* **110**, 283–294.
26. Bettler, B., Hofstetter, H., Mangala, R., Yokoyama, W. M., Kilchherr, R. & Conrad, D. H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7566–7570.
27. Collawan, J. F., Stangel, M., Kuhn, L. A., Esekogwu, V., Jing, S., Trowbridge, I. S. & Tainer, J. A. (1990) *Cell* **63**, 1061–1072.
28. Silverstein, S. C., Steinman, R. M. & Cohn, Z. A. (1977) *Annu. Rev. Biochem.* **46**, 669–722.
29. Wright, S. D. & Silverstein, S. C. (1986) in *Handbook of Experimental Immunology*, ed. Weir, D. M. (Blackwell, Oxford), pp. 41.1–41.14.
30. Silverstein, S. C., Greenberg, S., Di Virgilio, F. & Steinberg, T. H. (1989) in *Fundamental Immunology*, ed. Paul, W. E. (Raven, New York), 2nd Ed., pp. 703–720.
31. Hibbs, M. L., Bonadonna, L., Scott, B. M., McKenzie, I. F. C. & Hogarth, P. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2240–2244.
32. Ravetch, J. V., Luster, A. D., Weinshank, R., Kochan, J., Pavlovec, A., Portnoy, D. A., Hulmes, J. E., Pan, Y.-C. & Unkeless, J. C. (1986) *Science* **234**, 718–725.
33. Klickstein, L. B., Wong, W. W., Smith, J. A., Weis, J. H., Wilson, J. G. & Fearon, D. T. (1987) *J. Exp. Med.* **165**, 1095–1112.
34. Borish, L., Mascali, J. J. & Rosenwasser, L. J. (1991) *J. Immunol.* **146**, 63–67.