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IgE-binding Molecules on Human Langerhans Cells

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We have recently demonstrated that normal human Langerhans cells are able to bind IgE. The study of IgE-binding molecules on normal LC led to the characterization of three distinct structures able to bind IgE, viz. the low affinity receptor for IgE, FcεR2/CD23, the so-called IgE-binding protein εBP which is the human *homologous* of the murine Mac-2 antigen, and finally the high affinity receptor for IgE, FcεRI. In this review, we summarize the most recent data on these structures and their putative physiological relevance is discussed with regard to the atopic disease. *Key words: Langerhans cells; Receptors for IgE; Atopic disease.*

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IgE-molecules play a central role in allergic reactions known as immediate hypersensitivity or type I reactions. The cellular mechanisms triggered by IgE-binding to receptors and/or the allergen-binding to IgE, i.e., crosslinking of IgE, depend on the interaction of IgE with specific receptors, i.e. the type of receptor involved on the surface of effector cells.

Since the presence of IgE molecules has been described on human Langerhans cells (LC) in atopic dermatitis (AD), we focused our interest in the characterization of IgE-binding structures expressed on LC. This was done because we felt that these structures may represent a crucial element for a better understanding of the putative pathophysiological role of LC in AD and possibly as target for future therapeutical concepts. Very recently, we obtained evidence that in fact human LC express all three IgE-binding structures so far characterized in the human immune system, designated high affinity receptor (FcεRI), low affinity receptor (FcεRII) and IgE-binding protein (εBP). I will next summarize the most recent data concerning these structures on LC and discuss their significance for the pathophysiology of AD.

1. The low affinity receptor for IgE, FcεRII/CD23

The low affinity ($Ka = 10^7 M^{-1}$) receptor for IgE, FcεRII/CD23, was initially described on B-lymphocytes, and subsequently on other leukocytes, e.g. monocytes, macrophages, eosinophils, platelets, NK-cells and antigen-activated T cells (most aspects of FcεR2/CD23 are reviewed in 1). Since it has been reported that IgE-binding on LC isolated from skin of AD is inhibited by BB10 (2), a monoclonal antibody reacting with the FcεRII on eosinophils, but not by anti-CD23 reagents, it was assumed that the IgE-binding structure on LC was identical to that of eosinophils and macrophages but different from the FcεRII/CD23 expressed on other leukocytes. Since, in contrast to CD23, the FcεRII on eosinophils has not so far been cloned, the doubt may remain about the existence of a distinct FcεRII on eosinophils. Furthermore, two anti-CD23 mAb, viz. 135 (3) and 3–5 (Capron M, personal communication) clearly react with the FcεRII on eosinophils and strongly suggest extended similarities between the two receptors.

1.1. Evidence for a constitutive expression of FcεRII/CD23 on Langerhans cells in situ

Recent observations provided evidence of a constitutive expression of FcεRII/CD23 on LC. Indeed, we and others could demonstrate, by the means of improved immunohistochemical or immunoelectron microscopic techniques, that resident LC actually express small amounts of FcεRII/CD23 (4, Bieber, unpublished data). This expression is hardly detectable on freshly isolated LC since they have to be prepared by proteolytic digestion of the epidermal tissue, which in turn cleaves FcεRII/CD23 at the cell surface of LC. LC-preparation methods under gentle conditions followed by flow cytometric analysis with anti-CD23 antibodies further confirm the low level of FcεRII/CD23 expression on normal LC. Moreover, as with B cells, LC rapidly lose their CD23 moieties presumably by an autoproteolytic cleavage while accompanied by the release of soluble forms of FcεRII/CD23 (see below). This observation should be interpreted in the context of the profound phenotypic and functional differentiation of LC after their isolation from the epidermal microenvironment (5). Hence, in the light of these findings, it is not astonishing that we were initially not able able to detect FcεRII/CD23 on isolated LC. However, we could show that cultured LC may be induced to re-express FcεRII/CD23 by IL-4 and/or IFN-g (6) and behave like the monoblastic cell line U937.

It is clear that in the epidermal compartment, LC are exposed to several cytokines, which exert a complex regulatory network. Thus, cytokines known to be produced by keratinocytes, viz. IL-1, IL-3, IL-6, GM-CSF and TNF-α, are all more or less antagonists of the effect of IL-4 and IFN-gamma (7).

Nevertheless, the observation of a constitutive expression of FcεRII/CD23 on resident LC implies the question of its classification according to the cloning of FcεRII/CD23 which revealed two subtypes FcεRIIa and FcεRIIb, differing only by six amino acids in the cytoplasmic domain (8). Further characterization is in progress in order to clarify this very crucial point.

1.2. Langerhans cells release IgE-binding factors (soluble CD23)

A distinct feature of FcεRII/CD23 is its ability to cleave into soluble unstable 33–37 kDa fragments which are subsequently cleaved into stable 25 kDa, also named IgE-binding factors since they are still capable of binding IgE molecules (most aspects of soluble CD23 are reviewed in 9). Recent data from

our laboratory clearly demonstrated that LC cultured in the presence of IL-4 or IFN-gamma produce significant amounts of IgE-binding factors. We could show that, in contrast to IL-4 which almost induces the surface expression of FcεRII/CD23 on LC, gamma-interferon clearly promotes the release of soluble CD23 moieties (10). Similar results were obtained with U937 cells and correlate to that reported in the literature on monocytes (11).

1.3. Possible fuuctioual significance of FcεRII/CD23 on LC

There is some evidence for an IgE-mediated antigen-presentation by LC in vivo and in vitro (12, 13). As mentioned, both species of FcεRII (FcεRIIa and FcεRIIb) differ in their cytoplasmic segment but are identical in their extracellular portion. This led to the speculation that only FcεRIIb is functional in the effector phase of allergy and parasitic infections (8). Involvement of FcεRII/CD23 on antigen-presenting function by B cells has been demonstrated recently (13, 14) but whether only FcεRIIa – and not FcεRIIb is capable of internalization after binding to IgE remains unclear. Therefore, the FcεRII/CD23 present on LC deserves further characterization in terms of functional significance, especially in the putative FcεRII-mediated internalization of IgE-antigen complexes.

Considerable amounts of sometimes conflicting data are now available concerning the biological activity of soluble CD23. This includes (i) a permissive effect of the 33 kDa fragments on the synthesis of IgE in an IL-4-independent activation stage, (ii) a growth factor-like activity of 25 kDa fragments in the differentiation of thymocytes, and (iii) a similar activity on early bone marrow myeloid precursor cells. Whether this last type of activity is of relevance for soluble CD23 fragment release by LC in the skin remains questionable, unless extra-thymic maturation occurs in human epidermis, as has been suggested in the mouse. However, since LC are able to migrate in regional lymph nodes, IgE-binding factors released in these areas may also play a role in the regulation of IgE synthesis. Indeed, it has been shown that, under defined experimental conditions, LC stimulate IL-4-producing T cells (15). Therefore, 33–37 kDa fragments locally released by LC may support the effect of IL-4 and amplify the positive signal for IgE synthesis. This aspect is of considerable interest with regard to the possible involvement of LC in the primary and secondary immune response to aeroallergens through the epidermis and other epithelia where LC are localized. Thus, so far, it remains unclear whether the sensitization to aeroallergens may occur not only by the upper airways but possibly through transepidermal penetration. Some observations based on differences in serum levels for specific IgE for allergens derived from house dust mite body and house dust mite faeces in asthmatic children and in children with AE suggest that sensitization to allergens from the mite body occurs via penetration in the skin (16).

Most remarkable, is the recent finding that soluble CD23 may affect the migration of monocytes (17). Although the exact mechanism of this activity remains to be elucidated, this effect seems to be of greatest relevance for resident LC. This is suggested by (i) the preferential release of sCD23 upon IFN-g stimulation of LC (10), and (ii) the inhibitory activity of IFN-g on the spontaneous migration of LC in skin organ cultures (18).

2. The endogenous β-galactoside specific soluble lectin εBP (IgE-binding protein)

IgE-binding protein (εBP) is a 31 kDa protein originally described on rat basophilic leukaemia cells (RBL) (most aspects of εBP are reviewed in 19). A cDNA coding for εBP showed that it lacks a classical signal peptide or transmembrane domain. Subsequently, it was demonstrated that εBP is highly homologous to (i) carbohydrate-binding protein 35 (CBP35), a mouse galactose-specific lectin found in fibroblasts, and (ii) Mac-2, a cell surface protein expressed mainly on murine thioglycollate-elicited peritoneal macrophages, macrophage cell lines, interdigitating dendritic cells, some epithelial cells, and, most important, on murine LC (20). A close relationship of εBP with the human lectin HL-29 was obtained from sequence data and confirmed that all these structures, i.e. εBP, Mac-2, CBP35 and HL-29, are very similar and belong to a group of endogenous soluble lectins with S-type carbohydrate recognition activity (21).

2.1.Human Langerhans cells express the IgE-binding protein, εBP

Very recently, using an antiserum named εBP1, directed against a synthetic peptide (FNPRFNENNRRVIVC) corresponding to the highly conserved (identical in rodents and human) stretch of a.a. 174– 188 of the carboxyterminal portion of εBP, we were able to detect this protein on CD1a-positive LC in cryosections of normal skin, both by immunohistochemistry and as on isolated LC by flow cytometric analysis (Wollenberg et al., submitted). The biochemical analysis of the εBP1-reactive material confirmed the presence of a protein with a molecular weight of 31 kDa consistent with εBP. Most interesting, the expression of εBP is clearly enhanced in lesional skin of AD when compared with normal skin where LC are hardly stained. This would correspond to the upregulation of the molecule observed in thioglycollate-elicited macrophages. Further characterization of human εBP revealed an interesting pattern of reactivity to IgE molecules, in that εBP binds murine IgE but not all forms of human IgE (22), unless treated with neuraminidase which unmasks εBP-ligand in IgE. Polyclonal IgE molecules in human patient serum bind differently to εBP, suggesting that human IgE molecules are heterologous in terms of sialylation. Hence, LC bind murine IgE but only distinct subtypes of human IgE molecules via εBP. The physiological role of this phenomenon and its significance for LC is currently under investigation.

2.2. Putative functions of εBP on Langerhans cells

At least four different functions have been suggested for εBP. Firstly, εBP may act as a third type of IgE receptor. As such, εBP could be associated with either FcεRI or FcεRII and enhance their affinity for IgE or εBP functions as an autonomous IgE receptor. Secondly, it has been evoked that released-εBP may be analogous to histamine-releasing factors. Thirdly, recent data on CBP35 revealed that Mac-2, CBP35 and εBP are identical to the major non-integrin laminin bind-

ing protein of macrophages (23). Therefore, as for FcεRII/CD23 LC express here a defined lectin with a putative role in interactions with the extracellular matrix. Finally, as an intracellular protein, εBP may bind to IgE after FcεR-mediated internalization and operate as a cytoplasmic and/or nuclear signalling molecule for an appropriate response to the detected level of IgE.

3. Human Langerhans cells express the high affinity receptor for IgE, FcεRI

Considering the sparse knowledge of the characteristics of the IgE-binding structures present on LC isolated from patients with AD (2), some of these aspects would fit in an hypothetical expression of FcεRI by LC, e.g., trypsin resistance and affinity for IgE and to a lesser extent for IgG. However, one would expect a constitutive expression of FcεRI on normal LC, something that has so far not been reported. Recently, using human IgE myeloma protein, we and others were able to demonstrate that normal human LC bind IgE molecules in situ as well as in vitro. This IgE-binding could not be inhibited by anti-FcεRII/CD23 or anti-εBP or anti-FcγR reagents, suggesting that normal LC exhibit a third IgE-binding structure which is actually responsible for the greatest part of their IgE-binding capacity (24).

Thus we investigated LC for the putative presence of the high affinity receptor for IgE, FcεRI, which is composed of a tetramer viz. an α-subunit (FcεRIα), a β-subunit FcεRIβ), and two γ-subunits (FcεRIγ) and has, as yet, only been described on mast cells and basophils (most aspects on FcεRI are reviewed in 25). The most convincing arguments for a FcεRI expression on human LC came from our recent finding that these cells react *in situ* as well as *in vitro* with monoclonal antibodies raised against the α-subunit of FcεRI. Furthermore, our biochemical analysis of anti-FcεRIα reactive moieties by immunoblot revealed a protein of approximately 50 kDa, which is consistent with FcεRIα. Finally, by the mean of a molecular biological approach with reverse transcriptase and polymerase chain reaction, using primers specific for either of the subunits of FcεRI, we were able to clearly demonstrate that human LC express the complete structure of FcεRI (26). Finally, very recently we could demonstrate that FcεRI expression is lost during the profound phenotypic and functional alteration of LC in vitro.

3.1. Possible functional significance of FcεRI on LC

Crosslinking of receptor-bound IgE molecules on mast cells and basophils leads to the release of mediators known as the mediators of anaphylaxis. Recently, it became evident that mast cells also represent potent sources of a series of cytokines which may profoundly interfere with the immune system (27). However, the exact mechanism of the release of these substances is obscure and may be linked to activation processes different from the classical crosslinking of FCεRI. In the case of a putative FcεRI on LC, only speculations are allowed, since very little is known about the secretory repertoire of this dendritic cell. If FcεRI on LC were to keep the same role of a signal transduction unit for a rapid exocytosis of various inflammatory mediators as on mast cells or basophils, then the involvement of FcεRI in antigen-internalization, processing and antigen-presentation would be rather questionable. On the other hand, it is not excluded that such a crosslinking could lead to the release by LC of adequate chemotactic mediators for mononuclear cells at the site of interaction, i.e. the epidermis or the dermis.

CONCLUSIONS

It has been suggested that IgE-bearing LC may play a crucial role in the pathophysiology of AD. However, one should keep in mind that (i) IgE-bearing LC are not specific for AD, although there is some correlation with an elevated IgE level (28, 29); and (ii) this is far from explaining all clinical forms of AD. Assuming that IgE-bearing LC may form an important link between type I and type IV hypersensitivity reaction in AD, it is worthwhile to further characterize the structures responsible for IgE-binding on these cells. A series of observations indicate that epidermal LC express all three IgE-binding structures characterized so far, viz. the high affinity receptor (FcεRI), the low affinity receptor (FcεRII/CD23) and the 31 kDa IgE-binding protein (εBP, Mac-2, CBP35). Each of these molecules displays a distinct functional and therefore pathophysiological role which may be of crucial importance for a better understanding of the mechanisms leading to skin lesions in AD and the putative involvement of LC in the regulation of IgE synthesis.

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REFERENCES

1. Gordon J, Flores-Romo L, Cairns JA, Millsum MJ, Lane PJ, Johnson GD, Maclennan IC. CD23: a multi-functional receptor/lymphokine? Immunol Today 1989; 10: 153–157.
2. Bruynzeel-Koomen C, van-der-Donk EM, Bruynzeel PL, Capron M, de-Gast GC, Mudde GC. Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. Clin Exp Immunol 1988; 74: 137–142.
3. Grangette C, Gruart V, Ouaissi MA, Rizvi F, Delespesse G, Capron A, Capron M. IgE receptor on human eosinophils (FcεR2). Comparison with B cell CD23 and association with an adhesion molecule. J Immunol 1989; 143: 3580–3585.
4. Torresani C, Manara GC, Ferrari C, De Panfilis G. Immunoelectron microscopic characterization of a subpopulation of freshly isolated epidermal Langerhans cells that reacts with anti-CD23 monoclonal antibody. Br J Dermatol 1991; 124: 533–537.
5. Romani N, Lenz A, Glassel H, Stossel H, Stanzl U, Majdic O, Fritsch P, Schuler G. Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. J Invest Dermatol 1989; 93: 600–609.
6. Bieber T, Rieger A, Neuchrist C, Prinz JC, Rieber EP, Boltz-Nitulescu G, Scheiner O, Kraft D, Ring J, Stingl G. Induction of Fc epsilon R2/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and gamma interferon. J Exp Med 1989; 170: 309–314.
7. Bieber T, Rieger A, Luger T, Stingl G. Modulation of FcεR2/CD23 expression on normal human epidermal Langerhans cells by keratinocytes-derived cytokines. 7th International Congress of

Immunology, Gesesellschaft für Immunologie (ed). Fischer Verlag, 1989; 589.
8. Yokota A, Kikutani H, Tanaka T, Sato R, Barsumian EL, Suemura M, Kishimoto T. Two species of human Fc epsilon receptor II (Fc epsilon RII/CD23): tissue-specific and IL-4-specific regulation of gene expression. Cell 1988; 55: 611–618.
9. Delespesse G, Sarfati M, Hofstetter H. Human IgE-binding factors. Immunol Today 1989; 10: 159–164.
10. Bieber T, Delespesse G. Gamma-interferon promotes the release of IgE-binding factors (soluble CD23) by human epidermal Langerhans cells. J Invest Dermatol 1991; 97: 600–603.
11. Delespesse G, Sarfati M, Peleman R. Influence of recombinant IL-4, IFN-alpha, and IFN-gamma on the production of human IgE-binding factor (soluble CD23). J Immunol 1989; 142: 134–138.
12. Reitamo S, Visa K, Kahonen K, Kayhko K, Stubb S, Salo OP. Eczematous reactions in atopic patients caused by epicutaneous testing with inhalant allergens. Br J Dermatol 1986; 114: 303–309.
13. Mudde GC, Van-Reijsen FC, Boland GJ, de-Gast GC, Bruijnzeel PL, Bruijnzeel-Koomen CA. Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. Immunology 1990; 69: 335–341.
14. Kehry MR, Yamashita LC. Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE-dependent antigen focusing. Proc Natl Acad Sci USA 1989; 86: 7556–7560.
14. Pirron U, Schlunck T, Prinz JC, Rieber EP. IgE-dependent antigen focusing by human B lymphocytes is mediated by the low-affinity receptor for IgE. Eur J Immunol 1990; 20: 1547–1551.
15. Hauser C, Snapper J, O'Harra W, Paul W, Katz S. T helper cells grown with hapten-modified cultured Langerhans cells produce interleukin 4 and stimulate IgE production by B cells. Eur J Immunol 1989; 19: 245.
16. Carswell F, Thompson S. Does natural sensitization in eczema occur through the skin? The Lancet 1986; 11: 13.
17. Flores-Romo L, Cairns JA, Millsum MJ, Gordon J. Soluble fragments of the low-affinity IgE receptor (CD23) inhibit the spontaneous migration of U937 monocytic cells: neutralization of MIF-activity by a CD23 antibody. Immunology 1989; 67: 547–549.
18. Zierke J, Bieber T. Inhibition of the spontaneous migration of human epidermal Langerhans cells in organ cultures by interferon gamma. J Invest Dermatol 1991, 96: 1013.
19. Liu FT. Molecular biology of IgE-binding protein IgE-binding factors, and IgE-receptors. Crit Rev Immunol 1990; 10: 289–306.
20. Haines KA, Flotte TJ, Springer TA, Gigli I, Thorbecke GJ. Staining of Lagerhans cells with monoclonal antibodies to macrophages and lymphoid cells. Proc Natl Acad Sci USA 1983; 80: 3448–3452.
21. Gitt MA, Barondes SH. Evidence that a human soluble β-galactoside-binding lectin is encoded by a family of genes. Proc Natl Acad Sci USA 1986; 83: 7603–7607.
22. Robertson MW, Albrandt K, Keller D, Liu FT. Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. Biochemistry 1990; 29: 8093–8100.
23. Woo HJ, Shaw LM, Messier JM, Mercurio AM. The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). J Biol Chem 1990; 265: 7097–7099.
24. Bieber T. Mise en évidence de 3 structures distinctes fixant les IgE sur les cellules de Langerhans humaines. Ann Dermato Venereol (Paris) [in press].
25. Ravetch JV, Kinet JP. Fc receptors. Annu Rev Immunol, 1991; 9: 457–492.
26. Bieber T, de la Salle C, Wollenberg A, Chizzonite R, Hakimi J, Ring J, Hanau D, de la Salle H. Constitutive expression of the high affinity receptor for IgE (FcεRI) on human Langerhans cells. J. Exp. Med 1992 [in press].
27. Gordon JR, Burd PR, Galli SJ. Mast cells as a source of multifunctional cytokines. Immunol Today 1990; 11: 458-462.
28. Bieber T, Dannenberg B, Prinz JC, Rieber EP, Stolz W, Braun-Falco O, Ring J. Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the IgE serum level. J Invest Dermatol 1989; 93: 215–219.
29. Bieber T, Braun-Falco O. IgE-bearing Langerhans cells are not specific for atopiceczema but are found in inflammatory skin diseases. J Am Acad Dermatol 1991; 24: 658–659.