Regulation of IgE Synthesis by CD23/CD21 Interaction

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Abstract

At least two **cell-derived** signals have been shown to be necessary for the induction of immunoglobulin isotype switching in B cells. The first signal is given by either of the soluble lymphokines interleukin (IL)-4 or IL-13 which induce germline ϵ transcript expression, but alone is insufficient to trigger secretion of IgE. The second signal is provided by a physical interaction between B cells and activated T cells, basophils and mast cells, and it has been shown that the CD40/ CD40L pairing is crucial for mediating IgE synthesis. In HIGM1 syndrome, which is characterised by greatly decreased levels of IgG, IgA and IgE, there are mutations in CD40L resulting in a completely **non-functional** extracellular domain. CD40L is therefore playing a central role in Ig switching. Amongst the numerous pairs of surface adhesion molecules, the CD23/CD21 pair seems to play a key role in the generation of IgE. The CD23 molecule is positively and negatively regulated by factors which increase or decrease IgE production, respectively. Antibodies to CD23 have been shown to inhibit IL-4-induced human IgE production in vitro and to inhibit **antigen-specific** IgE responses in a rat model, in an **isotype-selective** manner. CD23 interacts with CD21 on B cells, preferentially driving IgE production. CD23 recognises two main epitopes on the CD21 molecule. One region consists of short consensus repeat sequences (SCRs) 1–2 and the other of SCRs 5–8. In the latter region Asn370 and Asn295 are critical in the interaction with the lectin CD23. Therefore, a restricted number of cytokines and surface molecules seems to selectively regulate human IgE synthesis.

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CD23 is a type II membrane glycoprotein containing four highly conserved and two partially conserved cysteines with C-type lectins [1]. A family of proteins displaying this lectin motif binds specific carbohydrates in a Ca^{2+} -depend-ent fashion [2]. The binding of IgE to CD23 is calcium dependent [3] as is the interaction between CD23 and CD21 [4]. The specific sugar(s) recognised by CD23 on its ligands has yet to be characterised. However, fucose-1-phosphate was found to inhibit binding of IgE to CD23 [5] and the CD23/CD21 interaction [4].

The CD23 molecule is expressed on a wide variety of cells including B cells, T cells, a subset of thymic epithelial cells, follicular dendritic cells, Langerhans cells, mono-cytes, eosinophils, platelets, and Epstein-Barr virus (EBV)- containing nasopharyngeal carcinoma cells [5].

Interleukin (IL)-4 and IL-13, which increase CD23 expression, were also found to induce IgE production by normal B cells by causing IgE isotype switching [6, 7]. IL-4-induced IgE synthesis was blocked by interferon (IFN- γ), as well as by IFN- α , and prostaglandin E2 [8], factors which

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also inhibit the induction by IL-4 of CD23 on B cells [9]. Soluble (s)CD23 has been shown to augment ongoing spontaneous IgE production by B cells derived from atopic individuals [10] and to act synergistically with a suboptimal concentration of IL-4 to induce IgE production by normal human B cells [8]. The importance of CD23 or its soluble fragments in the synthesis of human IgE in vitro was highlighted by the demonstration that a specific subset of anti-CD23 monoclonal antibodies blocked IL-4-induced IgE production by normal B cells [8] as well as the spontaneous production of IgE by B cells from atopic patients [11]. Moreover, CD23 was shown to modulate the antigen-specific IgE response in an isotype-selective manner in vivo [12]. Physical interaction between T cells and B cells is a process known to be required for IgE production [13]. Therefore, one possible role for CD23 in IgE regulation would be to enhance the T cell-B cell interaction [14]. In support for such a role, we have found that certain anti-CD23 monoclonal antibodies inhibit T/B cell conjugate formation in vitro [15] and that this inhibition is restricted mainly to CD4⁺ T cells which form conjugates with B cells. Studies using inhibitory anti-CD21 antibodies as well as the binding of the CD23-liposomes to recombinant CD21-transfected cells revealed that CD23 binds to a subtype of CD21 [16], a molecule that had been previously identified as the receptor for the EBV and as the complement receptor-2. CD21 is expressed on B cells, follicular dendritic cells and T cells. Anti-CD21 antibodies, like anti-CD23 antibodies, were found to decrease T/B conjugate formation [15], as would be expected if CD23/CD21 pairing has a role in the T cell/B cell interaction. Thus CD23 appears to be another molecule involved in the T cell/B cell interaction. But the particular characteristic of the CD21/CD23 pair, when compared to the others, is that this pairing controls IgE synthesis in an isotype-specific manner. Engagement of CD21 on B cells by some anti-CD21 monoclonal antibodies increased IL-4-induced IgE production, as did treatment with recombinant sCD23 [16]. Molecular analysis revealed that triggering of CD21 increased the IL-4-induced germline ϵ transcription levels and had a synergistic effect on the expression of the ϵ transcript induced by T cells [17]. On the basis of the observations that triggering of CD21 by either anti-CD21 antibodies [16] or by recombinant sCD23 enhances IgE production in both T-cell-dependent and T-cell-independent systems [17], and the reported induction of CD23 on T cells by IL-4 and allergen [18], one can speculate that in allergic individuals, T-cell-associated CD23 could interact with B-cell-associated CD21 leading to an increase in IgE production. Conversely, the T cell/B cell interaction leading to IgE production would not occur in normal individuals due to the absence of CD23 expression on T cells.

In order to determine the site of interaction of CD23 on the CD21 molecule, we used CD21 mutants bearing deletions of extracytoplasmic short consensus repeat sequences (SCRs). Interestingly, purified recombinant CD23 incorporated into fluorescent liposomes [4] binds to SCRs 5-8 on CD21, but SCRs 1-2 also seem to participate in the interaction with CD23. By mutating individually the three asparagine present on SCRs 5-8 (positions 295, 370 and 492), we strongly reduced the binding of CD23-liposomes close to the level of glycophorin-liposomes with mutated Asn295 and Asn370 but not Asn492 and by this means demonstrated the recognition of sugar moieties by CD23 in this region of CD21 [19]. The interaction of CD23

with SCRs 1-2 does not involve sugars [19]. An EBV peptide reported to bind to CD21 was found to decrease CD23-liposome binding to CD21. Moreover, this EBV peptide was able to decrease IL-4-induced IgE and IgG4 production from human mono-nuclear cells. In contrast, IgM and IgA production were increased. This finding is in line with our previous observation using anti-CD23 antibodies which blocked IgE production by blocking CD23/CD21 interaction. A C3 peptide reported also to bind to CD21 on SCR 3 was unable to block CD23 binding to CD21 and also unable to inhibit IgE production [Henchoz-Lecoanet, submitted]. In conclusion, the CD23/CD21 interaction controls IgE production more selectively than CD40/CD40L. CD23 is a surface molecule which can be cleaved and can lead to the release of soluble fragments bearing biological activities, at least in vitro. This molecule can therefore be considered as an adhesion molecule increasing cell-cell contacts and also as a cytokine, membrane bound then soluble. A better understanding of the key players in IgE regulation will increase our future chances for more effective medicines.

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