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IgE regulation in CD23 knockout and transgenic mice

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Discovery of CD23

The low-affinity IgE receptor was first noted by Lawrence et al. (1) while looking for Fc receptors for immunoglobulins other than IgG. An IgE myeloma protein was found to bind human lymphocytes as well as IgG (1), and monomeric forms of IgE bound to lymphocytes with a low affinity. In contrast, F(ab')₂ anti-human Fab-aggregated radiolabeled myeloma IgE could bind with a higher affinity to peripheral blood lymphocytes. Binding of IgE-coated erythrocytes (2, 3) and ¹²⁵I-labeled IgE was later found to involve interaction with Fc_εRII, which is a 47-kDa polypeptide cell-surface molecule that is highly susceptible to proteolysis (4). CD23 was originally identified as the Epstein-Barr virus (EBV) cell-surface antigen because B cells activated with EBV express the polypeptide. Cloning studies have revealed that CD23 and Fc_εRII are the same protein (5, 6).

Structure of CD23

Membrane form

CD23 is a type II integral membrane glycoprotein with a carboxy terminal C-type lectin head (7) which binds its ligand, IgE, in a calcium-dependent manner (8). CD23 is not a member of the immunoglobulin superfamily, a fact which makes it unique among Fc receptors. Cloning studies have revealed that a single polypeptide chain is sufficient to reconstitute IgE binding and CD23 expression on both human (9) and mouse (10) cells. Human CD23 and mouse CD23 are highly homologous, although at present the only ligand known for mouse CD23 is IgE. The stalk region of CD23 is composed of 21 amino-acid repeat domains (9, 11)

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that mediate oligomerization (see below). Human CD23 has three such repeats, and mouse CD23 has four. Gould et al. (12) demonstrated that, in common with tropomyosin, CD23 has a heptad amino-acid repeat that places leucines on one face of the molecule. This type of pattern is characteristic of molecules that form an α -helical coiled coil, and from these studies, CD23 was predicted to exist on the cell surface as a trimer, with the α -helical coiled-coil stalk causing the oligomerization (13). In 1993, Dierks et al. (14) mutated three of four of the 21-amino-acid repeat regions in the mouse CD23 stalk region and found that the mutated CD23 bound IgE with a single, low affinity. Since the mutated CD23 no longer cross-linked to a trimer, this finding further supported the oligomerization model. Similar cross-linking studies with human CD23 allowed the same conclusion (15).

A number of deletion and chimeric constructs have been made to determine the importance of oligomerization in IgE binding. The characteristic dual affinity seen for the IgE-CD23 interaction (14) was hypothesized to result from interaction with an oligomer rather than a monomer, and support for this was seen in the fact that sCD23 is monomeric and exhibits a single low affinity (16). Recently, researchers made a chimeric sCD23 consisting of the entire extracellular region of CD23 linked to a modified leucine zipper (17). This chimeric construct remained oligomeric even in the soluble form and exhibited IgE binding that was at least comparable to membrane-bound CD23. Indeed, the strength of binding was sufficient to cause inhibition of binding to the high-affinity Fc ϵ RI on mast cells (17). Thus, the stalk of CD23 primarily plays the role first suggested by Beauvil et al. (13); that is, it mediates the formation of an α -helical coiled-coil, allowing interaction with the IgE at more than one site (13). Thus, the high-affinity binding seen for oligomeric CD23 actually reflects an avidity situation due to this dual interaction.

Soluble form

Smaller forms of CD23 have been observed since its discovery in both man and mice. A soluble form of CD23, sCD23, is produced by proteolytic cleavage of the membrane form (18) or cleavage of newly synthesized intracellular forms (19). IgE interacts primarily with the oligomeric form of CD23 (14), and this form is less susceptible to proteolysis (2). The protease responsible for cleaving membrane CD23 has not yet been identified. Recent data indicate that an as yet unidentified metalloprotease is responsible for the cleavage (20, 21). The 37-kDa protein is cleaved from the

human carboxy terminus at amino-acid residue 81 (22). Letellier et al. (22) showed that the 37-kDa fragment is further degraded into molecules of 33, 29, 25, and 12 kDa. All human soluble forms (except the 12-kDa fragment) can bind IgE, albeit with low affinity (23, 24). Murine soluble forms are cleaved into fragments of 38, 35, 28, and 25 kDa (18), and murine sCD23 can bind IgE with low affinity only (16). The 38-kDa fragment is the only soluble form containing a N-linked glycosylation site (25).

Site of interaction between IgE and CD23

Monoclonal antibodies (mAb) against C ϵ 3 of human or murine IgE abrogate binding to CD23 (26, 27). The C ϵ 4 domain may also play a role in IgE binding because CD23 cannot bind IgE lacking C ϵ 4 or with C ϵ 3 alone (28). Bettler et al. (11) used deletion mutants to determine that a 123-amino-acid sequence in the lectin domains binds to a region homologous to carbohydrate-binding domains of C-type lectins and mediates IgE binding. Richards & Katz (8) found that the membrane CD23-IgE interaction is not inhibited by carbohydrates, and that CD23 binds IgE that has been treated with endoglycosidase (28). This finding indicates that the C-type lectin of CD23 interacts with protein on IgE rather than carbohydrates. However, it remains possible that interaction of CD23 with alternate ligands (see below) may involve carbohydrate

Other ligands for CD23

Within the last few years, two new ligands for CD23 have been revealed. Pochon et al. (29) showed that two B-cell myeloma lines, germinal center B cells, and some T cells bound CD23 incorporated into liposomes. Inhibitor studies with anti-CD23 mAb blocked these interactions, and through the use of a series of anti-B-cell antibodies, the interaction was shown to be with CD21 (30). CD21 is a phosphoprotein that is part of the B-cell receptor complex through its interaction with CD19 (31). It is expressed on lymphocytes, follicular dendritic cells (FDC), and pharyngeal epithelial cells, is a receptor for IFN- α and EBV, and is the complement receptor 2 (CR2) (32). There is strong evidence that the interaction of CD21 and CD23 can influence B-cell activation (33). Early work has also suggested that CD23 could act as an adhesion molecule; however, additional studies did not support this concept (34).

Lecoanet-Henchoz et al. (35) incubated CD23 liposomes with human blood monocytes which lack CD23 and IgE, and saw binding/aggregation of the cells in a rosette formation.

The new ligand was identified by running monocyte extracts over an affinity column coupled with recombinant sCD23. Western analysis identified CD11b as the protein bound to the column. Antibodies against CD11b and CD11c blocked monocyte interactions with the CD23 liposomes. Both CD11b and CD11c complex with CD18, and interactions of these complexes with CD23 functionally increase nitric oxide, H_2O_2 , IL-1b, IL-6, and TNF- α release from macrophages [36, 37]. Determination of new CD23 ligands supports the concept that CD23 regulates immune responses of cell types other than lymphocytes.

Lessons from CD23 knockout and transgenic mice

IgE regulation in CD23^{-/-} mice

Several groups have analyzed the role of CD23 *in vivo* in the murine model. Yu et al. [38] examined CD23^{-/-} animals on a C57Bl/6 background and reported an increase in antigen-specific IgE levels in response to thymus-dependent antigens. On the basis of these data, Yu et al. [38] proposed that CD23 acts as a negative regulatory feedback signal. Thus, allergic responses in the absence of CD23 would be more severe. However, Stief et al. [39] and Fujiwara et al. [40] also used CD23-deficient animals, albeit on a different background (BALB/C $\times$ 129J), and found no significant differences with respect to IgE levels in animals immunized with alum-antigen or parasite infection models. Fujiwara et al. [40] did find that the augmentation of the immune responses that is seen in the presence of IgE immune complexes, initially reported elsewhere [41], was absent in the CD23^{-/-} animals.

Evidence for the importance of CD23 in allergic disease has also come from studies of airway hyperresponsiveness (AHR) in CD23^{-/-} animals. Haczku et al. [42] used the CD23^{-/-} animals from Fujiwara et al. [40] and found that antigen-specific IgE was significantly enhanced in actively sensitized mice. This result is in line with Yu et al.'s study [38] already mentioned. In addition, the CD23^{-/-}-sensitized animals exhibited AHR and airway eosinophilia [42] when compared to parental controls. This work is supported by another report by Cernadas et al. [43], who also used the AHR model to show an increase in inflammation after sensitization and challenge with allergen. Cernadas et al. [43] found that anti-CD23 mAbs would attenuate the AHR, providing that intact mAb was used, suggesting that a CD23 signaling pathway was involved. In addition, they confirmed the findings of Haczku et al. [42] in that CD23^{-/-} mice

exhibited enhanced AHR compared to the parental controls. Thus, these studies support the concept that CD23 acts as a negative regulator of the allergic response. The CD23^{-/-} animals were further described to have an increase in lymphocyte activation, and an increase in the number of eosinophils detected in BAL fluid, with no changes observed in the numbers of neutrophils or lymphocytes. Also consistent with CD23 acting as a negative regulator, the deficient mice exhibited an increase in IgE levels after the sensitization and challenge with OVA over the PBS and wild-type controls.

IgE regulation in CD23 transgenic mice

The second model investigating the role of CD23 in IgE regulation *in vivo* involved CD23 transgenic animals, which overexpress CD23 on various cells types depending on the promoter used. Texido et al. [44] were the first group to develop a transgenic animal on the Thy-1.2 promoter/IgH enhancer with direct overexpression of membrane CD23 primarily on T cells. These investigators also made a soluble CD23 transgenic animal. Both animals were developed on the C57Bl/6XCB6 F₁ background. No discernible phenotype was seen with the sCD23 transgenic, other than enhanced sCD23 serum levels. Lymphocyte development was not affected in either of the genetically engineered strains, as determined by FACS analysis of splenocytes or immunohistochemistry. However, the membrane CD23 transgenics did exhibit significantly decreased IgE and IgG1 responses to *Nippostrongylus brasiliensis* and anti-IgD immunization, but not to antigen/alum. Eosinophilia was equally pronounced in the mCD23 transgenics and controls during the secondary *N. brasiliensis* response, indicating that the infection was efficacious in both transgenics and controls. Thus, the overexpression of CD23 in this background caused a reduction of IgE and IgG1 with respect to two IgE-induction models, with no difference observed in basal levels of IgE. Texido et al. [44] suggest that their results support the hypothesis that increased membrane CD23 is responsible for modulation of Th2-type responses.

The newest transgenic construct was generated on a (BALB/c $\times$ C57Bl/6) F₁ background, the murine MHC class I promoter (H-2Kb)/Ig heavy chain enhancer being used to limit CD23 expression to lymphocytes [45]. FACS analysis revealed that CD23 is overexpressed on both B and T cells in each of the founder lines tested with normal percentages of B and T cells in the spleens. Basal levels of IgE, IgG1, and all other isotypes were slightly suppressed compared to controls. These new transgenics exhibited a more impressive

suppression of IgE and IgG1 responses than did the Thy1.2 promoter transgenics in response to several IgE-inducing systems. One founder line in particular, M21, was not responsive to antigen/alum stimulation compared to their littermate controls with respect to IgE kinetics. IgE production was diminished in the other founder lines, M11 and M1, but not as impressively as in the M21 line. Suppressed IgE responses were also observed in response to helminth infections and anti-IgD stimulation in all three founder lines. Suppression was significant ($P < 0.05$) kinetically with regard to IgE production in the transgenics (45). The other Ig isotypes demonstrated a trend toward suppression, but the values were not significant. Serum IgE half-life values were normal in these animals. That finding in combination with the decreased IgE AFC formation observed indicated that IgE synthesis was affected (45).

In vitro studies with purified B cells, unstimulated or primed, were not able consistently to reproduce the suppression observed *in vivo*. Therefore, adoptive transfer studies were undertaken to determine whether the lymphocytes themselves or another potential background cell was responsible for the mechanism of suppression. Whole splenocytes were transferred from transgenic to control and from control to transgenic mice, with the recipient mice

having received 600 rad of ^{125}Cs 24 h before transfer. When IgE and IgG1 data were gathered from serum after injection with antigen/alum, the results clearly indicated a background cell type as being important for suppression of IgE, and not the lymphocytes (46). This conclusion was based on the finding that normal lymphocytes transferred to transgenic hosts exhibited the suppressed phenotype. One potential candidate for mediation of the suppression is the FDC, which normally expresses CD23 in mice (47). Current studies with purified FDC from the transgenics further support the claim that this cell type is involved in the suppression mechanism.

Thus, in summary, studies with $\text{CD23}^{-/-}$ and transgenic mice have supported the hypothesis that CD23 acts as a negative regulator for the allergic response. The importance of IL-4 and IL-13 for the induction of the allergic response has been realized for some time. The emerging studies with CD23 indicate that these cytokines induce both the IgE important for the allergic response and CD23, which ultimately modulates this same response. The studies further indicate that the development of protocols to enhance CD23 levels *in vivo* may be an effective new method to inhibit IgE production, and therefore IgE-mediated disease.

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