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Humoral Response Suppression Observed with CD23 Transgenics

Margaret E. Payet, Elaine C. Woodward, and Daniel H. Conrad

CD23, also known as the low affinity IgE receptor (FcεRII), has been hypothesized to have a role in IgE regulation. A new CD23 transgenic mouse was generated using the MHC class I promoter and IgH enhancer to further test the hypothesis that CD23 plays a role in the down-regulation of IgE. Study of three founder lines by FACS showed overexpression to varying extents on both B and T lymphocytes. No alterations in lymphocyte populations was observed. All three founder lines exhibited strong suppression of IgE in response to DNP-keyhole limpet hemocyanin/alum and *Nippostrongylus brasiliensis* infection compared with that in parental or littermate controls. The founder line exhibiting the highest level of suppression also was less susceptible to Ag-induced systemic anaphylactic shock. Overall, the data support the concept that enhancing CD23 levels can be used to suppress IgE-mediated disease. The mechanism involves decreased IgE synthesis, because the serum half-life of IgE was not altered in transgenics, and enzyme-linked immunospot analysis demonstrated lower IgE-producing cells stimulated by injection of anti-IgD.

Transgenics also exhibited significantly decreased IgG1 responses and exhibited lower levels of all Ig isotypes, although this was more variable in different founder lines. The Journal of Immunology, 1999, 163: 217–223.

The low affinity IgE receptor, FcεRII, was first observed by Lawrence et al. (1) in 1973. CD23 was initially described as a B cell activation marker (2), and cloning analysis later revealed that CD23 and FcεRII are the same protein (3). CD23 is unique among Fc receptors because it is not a member of the Ig superfamily. It is a member of the C-type lectin family, thereby requiring calcium for binding to IgE (4, 5). Two isoforms of CD23 are expressed (6), termed CD23a and CD23b. CD23b is expressed on a variety of hemopoietic cells in the human, including B lymphocytes (1, 7), follicular dendritic cells (FDC), T lymphocytes (8) eosinophils, platelets, macrophages, and NK cells (9). CD23a expression is limited to B cells and possibly FDCs (6), and since mice express primarily the CD23a isoform, expression is correspondingly limited (10, 11).

Membrane CD23 interacts with at least two additional ligands, in addition to IgE. These are CD21 (12) and CD11b/CD18 and CD11c/CD18 (13). Thus, not surprisingly, membrane CD23 has several roles, such as IgE binding, regulation of IgE synthesis, Ag processing (14), and B cell differentiation (2). Gene knockout and transgenic technology has been used to determine the function of CD23. The phenotype of CD23 knockout animals with respect to that of wild-type animals is somewhat controversial, Yu et al. (15) indicated that these animals have an enhanced IgE response to Ag/alum injections, whereas two other groups (16, 17) were unable to support these findings. Texido et al. (19) produced a transgenic strain in which CD23 transgenic expression was controlled by the Thy1 promoter, and these animals demonstrated some suppression of IgE production. In this study we report the characterization of a new transgenic model in which CD23 was over-expressed on both B and T lymphocytes by using the murine MHC class I promoter, H-2Kb, in conjunction with an IgH enhancer. Expression of the transgene was not seen on nonlymphoid tissue, such as heart and liver. The serum IgE half-life was similar in control and transgenic animals. These new transgenics were tested in vitro and in vivo for regulation of IgE as well as other isotypes in a variety of model systems. Transgenic animals consistently exhibited strongly suppressed IgE synthesis in Ag/alum immunization, *Nippostrongylus brasiliensis* (Nb) infections, and anti-IgD injection models. Sensitized animals also exhibited reduced sensitivity to systemic anaphylactic shock. Suppression of other Ig isotypes, especially IgG1, was also seen, although this effect was variable in different founder lines.

Materials and Methods

**Abs and reagents**

Mouse IgE from anti-DNP-ε-26 (18) and monoclonal anti-CD23 (B3B4 (19) or 2G8 (20)) were purified from appropriate ascites. B3B4 and 2G8 are both rat anti-mouse CD23 Abs that interact at a site similar to but not identical with the site on CD23 that binds IgE. The anti-leucine zipper M15, mouse IgG1, was a gift from Immunex (Seattle, WA) (21). B1E3 and R1E4 are both monoclonal rat anti-mouse IgE Abs that were purified from rat ascites as previously described (22). Rabbit B is a polyclonal anti-CD23 Ab purified from rabbit serum as previously described (20). Rabbit B was produced by injecting a rabbit with an engineered construct consisting of the extracellular region of CD23 (23) (EC-CD23) and was absorbed with mouse IgG before use. PE-anti-CD23 and FITC-anti-CD23 were obtained from PharMingen (San Diego, CA) for FACS analysis. FITC-anti-B220 (6B2) (24), anti-Thy1 (30H12) (25), anti-CD4 (GK1.5) (26), and 2.4G2 (27) were purified from nude mouse ascites and coupled to FITC using standard procedures. Coupling ratios (F/P) were always 6 or less. The anti-IgD mAbs H9/7 (28) and FF1–4D5 (28) were gifts from Dr. Fred Finkelman (University of Cincinnati, Cincinnati, OH). Recombinant CD40L and M15 were obtained from Immunix (Seattle, WA). IL-5 was purchased from R&D Systems (Minneapolis, MN), and baculovirus supernatant containing rIL-4 was a gift from Dr. William Paul (National Institutes of Health, Bethesda, MD). KLH and BSA (both from Sigma, St.
Louis, MO) were coupled to DNP as described previously (29). Coupling ratios for DNP:protein were determined as previously described (29), and preparations had a minimum ratio of 10.

**Transgenic preparation**

The pHE3′ vector was a gift from Steve Desiderio (Johns Hopkins University, Baltimore, MD). The preparation of this vector has been described previously (30); briefly, the vector is a puC18 base with a H-2Kd MHC class I promoter, a β-globin poly(A) signal for termination of the transcript, and an IgH enhancer to help limit expression of the transgene to lymphocytes. Support for this limited expression is seen in the use of this system for TCR (30) and btk (31) transgenics. CD23 cDNA was blunt ended and cloned downstream of the H-2Kd promoter and upstream of the β-globin splice site using the blunted SalI site; restriction analysis was used to determine correct orientation. An XhoI digest was used to remove puC18 sequences before microinjection into embryos. After gel isolation, the transgene construct was purified using a positively charged minicolumn (Qiagen, Valencia, CA), precipitated, and sent to the Transgenic Mouse Core Facility at the University of Pennsylvania, where it was microinjected into the male pronucleus of a (BALB/c × C57BL/6)F1 embryo. Tail DNA from progeny was analyzed by PCR; primer sequences were (sense) TACTCAGGATACTGGG and GATCGAGCTTCCCTT. If the transgene was present, a 1-kb piece was observed by gel electrophoresis. Due to the large intron size, the native CD23 was not amplified with these primers. Eight transgenic founder mice were initially identified and bred for further analysis. New pups were tagged for identification, with ear punches. The transgene was present, a 1-kb fragment was observed by gel electrophoresis if the transgene was present. Mice testing positive by PCR with either or both sets of primers or one set plus sCD23 ELISA (see below) were used in the experiments.

At 6–8 wk of age, positive progeny were sacrificed, and spleen cells were analyzed on a FACScan (Becton Dickinson) after double staining with FITC-6B2 or FITC-30H12 in combination with PE-B3B4 (each used at 10 μg/ml) for 30 min to 1 h on ice in the dark. Nonspecific binding was blocked using 10 μg/ml 2.4G2. Three founder lines were chosen for additional study based on the enhanced CD23 expression observed. The founder mice and their progeny were bred back to BALB/c mice to test responses of the transgene on a background of potential high IgE responses in BALB/c mice.

**Animals and cell isolation**

BALB/c and C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD) or The Jackson Laboratory (Bar Harbor, ME). All mice used in experiments were between 6–14 wk of age. Transgenic mice were maintained under pathogen-free conditions at Medical College of Virginia-Virginia Commonwealth University. B cells were isolated from disrupted spleens using a Percoll gradient as previously described (33, 34). Resting B cells were collected from the 66–70% interface, and activated B cells were collected from the 60–66% interface of a discontinuous Percoll gradient. Resting B cells (1 × 10⁶ cells/ml) were activated in vitro in a 24-well plate (Costar, Cambridge, MA) for 48 h with 50,000 U/ml IL-4, 5 ng/ml IL-5, 0.1 μg/ml CD40LT, and 0.1 μg/ml M15 at 37°C in a 5% CO₂ incubator. These activation conditions have previously been shown to be optimal (35). At the indicated times, cells were analyzed by FACS for surface markers.

**Ag immunization and Nb infection**

Methoxyflurane-anesthetized mice were immunized s.c. on day 0 and were challenged on day 14 with 100 μg of DNP-KLH in alum with heat-killed Bordetella pertussis (Sigma) as described previously (35). Mice were bled intracardially in the single time point analysis and by tail vein for the kinetic analysis. Nb infections were established by injecting 800 worms/200 μl of PBS s.c. on day 0 and were boosted (same dose) on day 20. Nb larvae were a gift from Dr. Thomas Huff (Medical College of Virginia-Virginia Commonwealth University) and were maintained as previously described (36). Mice were bled as described for the Ag/album immunizations, and serum was tested by ELISA.

**ELISAs**

The concentration of serum-soluble CD23 was determined by an ELISA assay previously described (23). Briefly, the coating Ab was 2G8, and the detection Ab was rabbit polyclonal anti-CD23 (rabbit B) followed by goat anti-rabbit HRP (Southern Biotechnology Associates, Birmingham, AL). The standard curve used the Escherichia coli-expressed EC-CD23 (23) protein as standard. The values shown represent duplicate samples determined at multiple dilutions.

Levels of IgE, IgG1, IgG2a, IgG2b, and IgG3 were determined by ELISA as previously described (35). Supernatants from B cells activated in vitro or serum from mice were analyzed for IgE as previously reported (20). Briefly, a pair of rat anti-mouse IgE monoclonals, B1E3 and R1E4, were used as the capture and biotinylated secondary Ab, respectively. Total IgG1, IgG2a, IgG2b, IgG3, and IgM were all determined using an unliganded primary goat anti-mouse Ab at 5 μg/ml and were detected using goat anti-mouse class-specific Ab coupled to alkaline phosphatase (all Abs are from Southern Biotechnology Associates). To detect Ag-specific IgG1 and anti-DNP IgE in serum from immunized mice, the ELISA plates were coated with 5 μg/ml KLH-DNP in ELISA BBS, and the rest of the ELISA protocol was completed in the same manner as that used for total IgG1.

**Anti-IgD immunization and ELISPOT**

Anti-IgD Abs, 100 μg of each H-2k and FFI–4D5 (both gifts from Dr. Fred Finkelman), and 200 μg of 2.4G2 were injected i.v. Eight days later, the mice were bled from the tail vein to test serum for IgE by ELISA. On day 9 spleens were removed, and activated B cells were isolated and used in an ELISPOT analysis using a modification of published protocols (37). ELISPOT Immulon 4 (Dynex, Chantilly, VA) plates were coated with 50 μg/ml B1E3 in 50 μl/well overnight at 4°C. A blocking solution of PBS with 5% PBS was added to the plate in a volume of 200 μl/well and incubated overnight at 4°C. The blocking solution was discarded, and a 150 μl/well medium was added for incubation for 10 min at room temperature. Splenic cells from anti-IgD-injected mice were added as follows: 10,000 cells were placed in the first well in complete B cell medium and diluted 1/2 across the plate at 150 μl of medium from wells 1–10. Wells 11 and 12 were used as medium alone blanks for the assay for each mouse tested. Each sample was run in duplicate. The cells were incubated for 5 h at 37°C in a 5% CO₂ incubator, then were discarded, and the plates were washed five times in ELISPOT wash solution (PBS with 0.25% Tween-20). The last three washes were for 5 min each, between which the wash solution was discarded. A 1/250 dilution of biotinylated R1E4 was added to the wells in blocking solution in 50 μl/well and incubated overnight at 4°C. Plates were washed five times as described above, and Streptavidin-AP (Southern Biotechnology) was added at 50 μl/well for 2 h at room temperature. ELISPOT substrate was added to the plates at 50 μl/well, and the plates were not moved until the agarose hardened. Spots were counted 24 h later using an inverted microscope. ELISPOT substrate consists of 1.25 ml of 3% low melting point agarose (Fisher Bioetch, Philadelphia, PA) in water; with 5 ml of 15 mg 5-bromo-4-chloro-3-indolyl phosphate (Sigma) in AMP buffer (15 mg MgCl₂-6H₂O, 10 μl of Triton X-405, 25 mg of Na₂HPO₄, and 9.6 ml of 2-amino-2-methyl-1-propanol, pH 10.25). The number of IgE-secreting cells was quantified by counting the number of blue spots per well and multiplying by the dilution factor, and was expressed as the number of AFC per million B cells.

**Serum IgE half-life**

The half-life of IgE was determined by injecting control and transgenic mice with an anti-DNP IgE mAb (18). Mice were injected intracardially with 10 μg of anti-DNP IgE. Each mouse was bled from the tail vein at 2, 4, 7, 12, and 15 h. Serum was assayed by ELISA for anti-DNP IgE at each time point as described for the ELISA.

**Systemic anaphylactic shock**

Using the Dombrowicz et al. protocol (38), control and transgenic mice were sensitized with 300 ng of Bordetella pertussis toxin (Sigma) in the tail vein and immunized i.p. with 405 μg of DNP-KLH in alum 48 h later. Mice were bled on day 20 to test serum for IgE by ELISA. One milligram of DNP-BSA in PBS with 2% Evans Blue dye (J. T. Baker Chemical, Phipsburg, N.J) was administered i.v. on day 21. The rectal temperature of the mice was followed for 70 min postinjection using an instrument (Yellow Springs Instruments, Yellow Springs, OH); readings were taken every 10 min. Temperature and leakage of the dye into tissue were used to determine whether mice were susceptible to systemic anaphylactic shock.
Expression of CD23 in new transgenic lines

The previously reported Thy1.1 promoter-driven CD23 transgenic exhibited increased CD23 expression on resting T cells, but overexpression of CD23 on B cells was demonstrated only after back-crossing the transgenic animals to CD23-/- animals (39). The new CD23 transgenics analyzed here used the murine class I promoter/IgH enhancer so that CD23 would be overexpressed on both resting B and T lymphocytes. An initial eight founder lines were obtained, and FACS analysis of splenic cells of progeny indicated that five of these founders exhibited overexpression of CD23. Data are presented from the three highest expressing founder lines (M1, M11, and M21) in this study. The highest level of CD23 expression was observed in the M21 line, and a representative example of CD23 overexpression is shown in Fig. 1, where overexpression is demonstrated on both T and B lymphocytes (Fig. 1, A and B). The overexpression remained evident on B cells even after activation with CD40L and IL-4 (Fig. 1, C–F). The use of the class I promoter suggested that transgene expression would be increased with IFN-γ; however, this did not prove to be the case. Increased CD23 expression due to IFN-γ either alone or in combination with CD40L (compare Fig. 1, D and E, and data not shown) was not observed. CD23 overexpression was also observed in the M1 and M11 lines, although the levels were, in general, less than that with M21 (data not shown). Overexpression was also evident in that transgenic mice exhibited increased soluble CD23 levels in serum; using a soluble CD23 ELISA, littermates had a sCD23 serum level of 127 ± 31, whereas transgenics had 5.4 ± 1 sCD23/ml, respectively. This characteristic was useful in confirming transgene expression in animals combined with PCR before use in experiments.

Expression on other tissues was also examined by immunohistochemical analysis. Heart and liver were examined and were found to be negative for expression of the transgene (data not shown). Thus, the data indicate that expression of the transgene is limited to primarily the lymphocyte compartment. This is consistent with other uses of this promoter enhancer system for analysis of TCR (30) and blk (31) transgenics.

Serum half-life of IgE does not differ from controls animals

CD23 overexpression could potentially result in absorption of IgE by transgenic CD23 to the extent that serum levels of IgE could be misleading (with respect to actual IgE synthesis) due to this absorption. Thus, the serum half-life of IgE was determined. DNP-specific monoclonal IgE (18) was injected into transgenic and littermate control animals, and serum levels of anti-DNP-IgE were determined by ELISA at different times. Analysis revealed that the half-life of IgE is 7.6 h in the transgenics and 9.7 h in the controls (Fig. 2). Both values were within the previously described 5- to 10-h half-life of serum IgE in mice (40).

Immune phenotype of transgenics: Ag/alum injection

Mice were immunized and boosted with DNP-KLH in alum plus heat-killed B. pertussis. Single time point analysis 9 days postbooster of serum by ELISA showed significant suppression of IgE and Ag-specific IgG1 in the transgenic animals compared with that in BALB/c parental controls or Thy1 promoter-driven CD23 transgenic animals (Fig. 3). The kinetics of the response were also examined using the same immunization protocol and bleeding the mice on the days indicated. Total IgE and DNP-KLH specific IgG1 were markedly suppressed over time in the M21 founder line (Fig. 4).

Immune phenotype of transgenics: Helminth infections

Nb is a helminth that normally induces high levels of IgE. Mice were infected with Nb larvae, and ELISA analysis revealed that both the M11 and M21 lines exhibited suppression of total IgE and IgG1 on day 14 postinfection compared with littermate controls (data not shown). Fig. 5 shows kinetic analysis of Nb-infected mice from both M11 and M21 founder lines. In the kinetic assays, mice were also given a secondary infection as indicated, the mice were bled during the course of the infection, and serum was tested...
for total IgE and IgG1 by ELISA. IgE was suppressed kinetically throughout the infection in both M21 and M11 lines. Note that no significant differences were observed in IgG1 in the M11 line, in contrast to the results of the single time point Nb experiment and the results seen with Ag/alum (Fig. 3).

Immune phenotype of transgenics: Anti-IgD injection

Two groups of mice were injected with the anti-IgD mAbs H8’/l and FF1–4D5 in combination with 2.4G2; stimulation with anti-IgD is known to give high IgE production (41). Analysis of serum IgE levels revealed reduced IgE production (Fig. 6A). In addition, spleen cells from anti-IgD-injected mice were examined by the ELISPOT protocol to determine whether the reduction in IgE correlated with a reduced number of IgE Ab-forming cells. The results are shown in Fig. 6B and demonstrate that the reduced serum IgE correlates with a reduction in IgE AFC.

Basal and immunized Ig levels of transgenics and controls

To determine whether overexpression of CD23 in these animals affected the basal levels of isotypes in the animals, serum from nonimmunized mice was also tested for IgM, IgG2a, IgG2b, IgG3, IgG1, and IgE by ELISA. A summary of the results is shown in
Table I. Basal and immunized isotype levels

<table>
<thead>
<tr>
<th>Type</th>
<th>IgE (ng/ml)</th>
<th>IgG1 (μg/ml)</th>
<th>IgM (μg/ml)</th>
<th>IgG2a (μg/ml)</th>
<th>IgG2b (μg/ml)</th>
<th>IgG3 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c (8)</td>
<td>107 ± 50</td>
<td>450 ± 247</td>
<td>1600 ± 640</td>
<td>195 ± 152</td>
<td>NT</td>
<td>104 ± 7</td>
</tr>
<tr>
<td>M11 (4)</td>
<td>23 ± 4</td>
<td>252 ± 69</td>
<td>1250 ± 76</td>
<td>16 ± 56</td>
<td>NT</td>
<td>78 ± 18</td>
</tr>
<tr>
<td>M21 (4)</td>
<td>2 ± 1</td>
<td>104 ± 16</td>
<td>489 ± 121</td>
<td>94 ± 17</td>
<td>NT</td>
<td>21 ± 4</td>
</tr>
</tbody>
</table>

Basal levels

<table>
<thead>
<tr>
<th>Immunized levels (Ag/alum)</th>
<th>BALB/c (4)</th>
<th>C57BL/6 (4)</th>
<th>M11 (8)</th>
<th>M21 (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (ng/ml)</td>
<td>439 ± 52</td>
<td>4325 ± 679</td>
<td>1422 ± 156</td>
<td>71 ± 13</td>
</tr>
<tr>
<td>IgG1 (μg/ml)</td>
<td>4561 ± 886</td>
<td>699 ± 154*</td>
<td>907 ± 123*</td>
<td>39 ± 7*</td>
</tr>
<tr>
<td>IgG2a (μg/ml)</td>
<td>692 ± 121</td>
<td>692 ± 121</td>
<td>209 ± 26*</td>
<td>75 ± 11</td>
</tr>
<tr>
<td>IgG2b (μg/ml)</td>
<td>76 ± 17</td>
<td>76 ± 17</td>
<td>75 ± 11</td>
<td></td>
</tr>
<tr>
<td>IgG3 (μg/ml)</td>
<td>89 ± 8</td>
<td>89 ± 8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All isotypes are decreased before and after immunization/infection. Mice are bled intracardially, either before or after alum/Ag immunization 9 days postbooster, and serum is tested by ELISA for IgM, IgG2a, IgG2b, and IgG3 as described in Materials and Methods. The numbers in parentheses indicate the number of animals tested in each group. All values are the result of at least three independent measurements and error bars are ± 1 SE. NT, not tested; *, p < 0.05 against BALB/c; and †, p < 0.05 against C57BL/6.

Overall, while there was a trend toward a decrease in basal Ig levels in both transgenic lines, the large variation in normal Ig levels resulted in a large SE and marginal significance. In addition, in selected immunization situations, other isotypes were examined. In general, the effects on all isotypes examined were suppressed, with the M11 values being intermediate between those in the M21 and BALB/c controls. The greatest suppression observed was with IgE, IgG1, and IgM. All other Ig levels were minimally affected, and IgA was not tested. Overall, the results are consistent with a general suppression of humoral immunity in these CD23 transgenics.

Protection from systemic anaphylactic shock

To determine whether the reduction in IgE levels was sufficient to protect the animals from anaphylactic shock, the transgenic line with the greatest IgE suppression (M21) was first sensitized to OVA and challenged on day 21 as described in Materials and Methods. A decrease in anaphylactic symptoms was observed. Namely, extrusion of the Evans Blue dye into tissues, as noted particularly with ear color, was greatly reduced in the transgenics (data not shown). Secondly, the littermate controls became unresponsive to stimuli and/or died as a result of the anaphylactic shock symptoms (three of four animals). To better quantitate the findings, the body temperature of the animals was determined every 10 min, and the values obtained are shown in Fig. 7. The body temperature of the littermate controls decreased from ~37 to 34°C, while the transgenics exhibited only a transient decrease in temperature of about 1°C and showed no decrease in activity levels. Protection from anaphylactic shock was also seen with the M1 transgenics in a similar manner (data not shown). This evidence indicates that the reduction of IgE by overexpression of CD23 in vivo can be sufficient to prevent generalized anaphylaxis.

Discussion

With the introduction of gene knockout and transgenic technology, the role of CD23 in vivo has been addressed by several groups. Yu et al. (16) prepared CD23 knockout mice on the C57BL/6 background and reported that immunization of CD23-deficient mice with thymus-dependent Ags resulted in an increased and sustained specific IgE level with normal germinal center formation. Immune responses to helminths was unaffected. This phenotype suggested that CD23 in vivo acts to negatively regulate IgE production. Stief et al. (17) and Fujiwara et al. also developed CD23-deficient mice and observed overall similar IgE responses compared with those of littermate controls. Heyman et al. (42) reported that IgE-Ag complexes could augment immune responses by interacting with CD23, and this augmentation was not present in the CD23−/− animals (16). Thus, while the general conclusion with the CD23−/− mice was that CD23 acted as a negative regulator for IgE production, the lack of CD23 resulted in a relatively modest phenotype.

Teixido et al. (39) developed a transgenic animal model in (C57BL/6 × CB6)F1 mice in which CD23 was overexpressed by the Thy1.1 promoter. These mice exhibited about a 50% suppression of the IgE response in the alum/Ag, anti-IgD, and Nb models, whereas IgG1 responses were similar to those of littermate controls. Expression of the transgene was mainly restricted to T lymphocytes; however, backcrossing onto the CD23−/− phenotype did demonstrate some expression on B cells. Overexpression of CD23 using the class I promoter/Ig enhancer in this new transgenic model resulted in clearly enhanced expression on both resting and stimulated B lymphocytes as well as T and non-B, non-T cells. This is directly seen in the comparison with the Thy1 transgenics shown in Fig. 3. Other transgenics prepared with this system, namely TCR (30) and blk (31), also reported primarily lymphocyte
expression. In that regard, sections of heart and liver from transgenics did not show any evidence of CD23 expression. It certainly remains possible that other tissues/cells can be induced to express the transgene, especially in the presence of IFN exposure; this aspect remains to be investigated.

The dramatic suppression of IgE responses in the various in vivo models tested lend further support to the concept that CD23 can regulate IgE levels. The mechanism of this suppression remains to be elucidated. Obviously, because both membrane and soluble CD23 levels are enhanced in the transgenic animals, the observed phenotype could be due to either membrane or soluble CD23 (or both). Because soluble CD23 transgenics had no observable phenotype (39), membrane CD23 is certainly favored as being responsible for the alterations observed. The current model of CD23 predicts that three monomers interact with each other to form a functional trimer on the cell surface. This model is based on the noted heptad repeat pattern found in the stalk region of the molecule (5) and chemical cross-linking studies (43). CD23 is cleaved by an as yet unidentified metalloprotease (44), and the cleaved monomeric product interacts with only a single low affinity with IgE (W. C. Bartlett et al., unpublished observations). Recently, Kelly et al. (23) found that a soluble CD23 oligomer with high affinity/avidity for IgE could be produced by attachment of a modified leucine zipper to the amino terminus of the stalk region of CD23. It will be interesting to determine whether transgenic animals producing this soluble oligomer have a phenotype similar to that of the membrane transgenics; such studies are in progress. The simplest explanation for the phenotype is that the CD23 overexpression absorbs secreted IgE, thereby removing it from circulation. Indeed, at least in humans, the anti-IgE therapy currently being used in clinical trials (reviewed in Ref. 45) appears to work primarily by removing IgE from the circulation. The lack of effect on serum IgE $1_{1/2}$ combined with the strong reduction in IgE AFC in mice injected with anti-IgD argues that the mechanism involves a reduction in IgE production. This laboratory also recently reported an in vitro model in which IgE production was inhibited by culture of B cells in the presence of CD23-expressing CHO cells (20). In both that study and ours, while the most dramatic inhibition involved IgE levels, some suppression of other Ig classes was also seen. Suppression of IgG1 was seen in all three transgenic lines (Fig. 3) in both alum/Ag-treated and Nb-infected mice (Figs. 4 and 5). However, we note that suppression of IgG1 was not evident in the M11 line in the kinetic analysis of Nb infection (Fig. 5). Although the reason for this variability is not clear at present, it can be determined whether variability in suppression is related to the background of the animals. Although the mice used in these studies had been bred back to BALB/c animals, the animals were used at generations 3–6; therefore, significant levels of C57 genes are used at generations 3–6; therefore, significant levels of C57 genes remained present. In any case, it is noted that the phenotype of the transgenics mimics a milder phenotype of CD21 $^{−/−}$ animals (46) in which an impaired B cell response to T-dependent Ags was observed. The inhibition of IgE and IgG1 combined with the lower levels of other Ig isotypes (Table I) suggests that overexpression of CD23 results in a general impairment of B cell differentiation/Ig production. We are currently examining germinal center formation and architecture in these mice and are especially interested in FDC function. CD23 is known to be expressed on FDC in mice (11), and high level expression of CD23 on FDC correlated with lower IgE levels in this study. In view of the capacity for CD23 and CD21 to interact (12), expression on FDC could potentially interfere with CD21 signaling on the B cell. Another possible explanation for the phenotype would be alterations in cytokine profiles resulting in CD23 transgenics having an enhanced Th1 response. We are currently investigating whether the cytokine profiles have been altered using Leishmania major infections, which require a Th1 response for clearance of the parasite.

Systemic anaphylaxis is a potentially fatal result of type I allergic disease. Anti-IgE therapy currently being tested in clinical trials is successful at removing IgE from the body for $\sim$3 mo, presumably via clearance of immune complexes by the reticuloendothelial system. Repeat injections are necessary, because IgE synthesis levels are not affected (reviewed in Ref. 45). It is noted, however, that anti-IgE treatment does appear to inhibit IgE synthesis in the mouse model (45, 47). Although successful at removing IgE, the anti-IgE Abs potentially cause serum sickness due to the non-human complementarity-determining region of the Ab. These studies suggest that an alternative therapy would be to develop protocols to elevate CD23 levels before B cell activation in view of the reduced IgE levels seen in these transgenics. While a general concern is inhibition of Ig responses, clearly the most dramatic effects are on IgE production. Therefore, the use of CD23, a native protein, to decrease IgE production remains an attractive strategy to control IgE production, providing that techniques to cause elevation of normal levels can be found. In this regard, the finding that metalloproteinase inhibitors can increase membrane CD23 levels and result in suppression of IgE production (48) further supports this concept.

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References


