

Effect of anti-IgE antibodies on IgE binding to CD23

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Human sera contain anti-IgE autoantibodies. Using a human B lymphoblastoid cell line (Wil-2WT cells) and monoclonal murine anti-IgE antibodies (BSW17 and Le27) we investigated a possible role of such anti-IgE antibodies. A 100-fold excess of monoclonal anti-IgE antibodies inhibited binding of ^{125}I labeled IgE to Fc ϵ RII on Wil-2WT cells. Further, both monoclonal anti-IgE antibodies dissociated surface bound IgE from Fc ϵ RII on Wil-2WT cells. However, BSW17 which does not trigger histamine release from human leucocytes, was much more effective in dissociating Fc ϵ RII bound IgE than Le27 which triggers histamine release. These results may suggest that naturally occurring IgG anti-IgE antibodies are able to inhibit binding of IgE to its receptor.

Key words: anti-IgE antibodies; CD23; Fc epsilon receptor II (Fc ϵ RII); human B lymphoblastoid cell line.

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Anti-IgE autoantibodies were described first in 1972 by Williams et al. (21). Several groups confirmed this observation and found that anti-IgE autoantibodies which were detected in human sera mainly belong to the IgG class (8, 12, 17, 19). Such IgG anti-IgE antibodies can be detected in normal as well as atopic sera (8). There seems to be also a correlation between elevated IgE levels and the concentration of anti-IgE antibodies in sera (16, 17). However, there is no clear evidence for a physiological or pathophysiological role of such anti-IgE antibodies *in vivo*. One potential role would be the inhibition of IgE binding to its receptor. Here we only analyzed the low affinity receptor for IgE (Fc ϵ RII or CD23) on a human B lymphoblastoid cell line and studied whether murine monoclonal antibodies against human IgE might be useful tools to investigate the potential role of naturally occurring IgG anti-IgE antibodies.

MATERIAL AND METHODS

Cell line

Wil-2WT cells (a human B lymphoblastoid cell line (14)) were maintained in RPMI 1640

medium (Gibco, UK) containing 10% heat-inactivated fetal calf serum (FCS, Biochrom KG, Berlin, FRG).

Immunoglobulins and antibodies

Human IgE was isolated from the serum of a myeloma patient (P.S.) and was a kind gift of Drs. Ishizaka (Baltimore, USA). We have described the generation of two types of monoclonal murine IgG₁ antibodies against human IgE recognizing different epitopes on Fc ϵ (4, 6, 11). The monoclonal antibody BSW17 recognizes the D_{ε1} region, while the monoclonal antibody Le27 recognizes the D_{ε2} region on human Fc ϵ (11).

Human IgG₁-lambda, IgG₂-kappa, IgG₃-kappa, IgG₄-lambda, IgA₁-lambda, IgA₂-lambda and IgM-kappa were obtained from Dr. Skvaril (Institute of Cancer Research, Tiefenanspital, University of Bern). Murine IgG₁ (anti-CD8; Clonab T8) was obtained from Biotest AG (Frankfurt, FRG) and murine IgG₂ (anti-CD3; BMA 030) was obtained from Behringwerke (Marburg, FRG). Anti-CD23 antibody (BB 10) was kindly provided by Dr. M. Capron (Lille, France (1)).

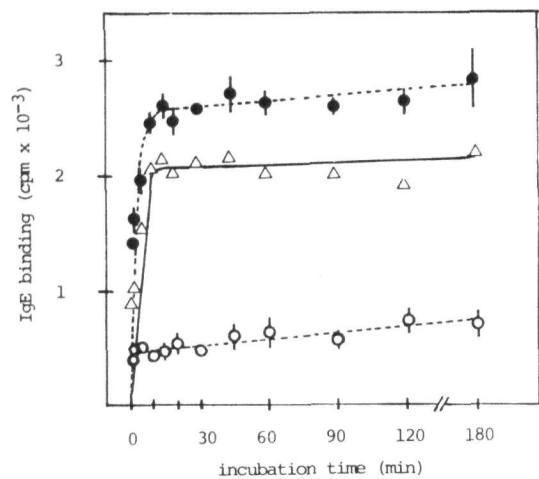


Fig. 1. ^{125}I -IgE binding to Wil-2WT cells. Total IgE binding (closed circles), nonspecific IgE binding (open circles) and specific IgE binding (open triangles).

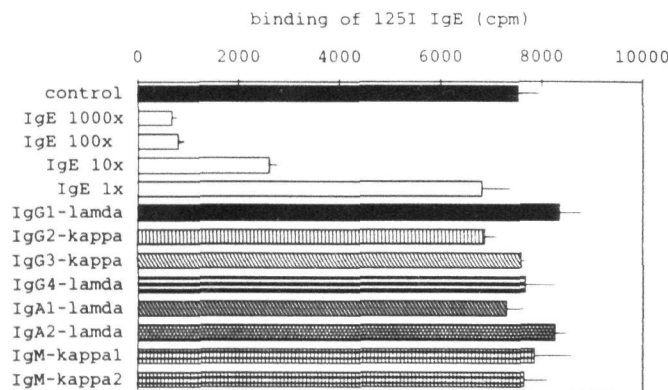


Fig. 2. ^{125}I -IgE binding to Wil-2WT cells. ^{125}I -IgE and a 100-fold excess of other classes of human immunoglobulins or different concentration of unlabeled IgE were incubated with Wil-2WT cells.

Measurement of ^{125}I -labeled human IgE binding to Wil-2WT cells

The binding of human IgE to Wil-2WT cells was measured by the method of Kulczycki (13) and Vander-Mallie (20) with slight modifications. Briefly, human IgE was labeled with ^{125}I using the chloramin-T method (5, 7). Wil-2WT cells were suspended in RPMI 1640 medium and 0.1 ml of the cell suspension (4×10^7 cells/ml) was incubated with 0.02 ml of ^{125}I -IgE (3.7 $\mu\text{g}/\text{ml}$; approximately 2×10^6 cpm/ml) in a total volume of 0.2 ml. For assessment of nonspecific binding, a 100-fold excess of unlabeled IgE (0.08 ml) was mixed with the cell suspension

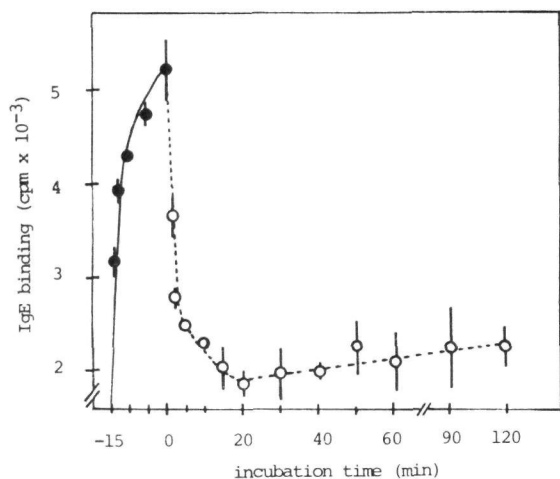


Fig. 3. Kinetics of cold competition with ^{125}I labeled IgE. Wil-2WT cell suspensions were incubated with ^{125}I -IgE for 15 min at 0°C . Then, a 100-fold excess of unlabeled IgE was added. This figure shows IgE binding curve (closed circles) and IgE dissociation curve (open circles).

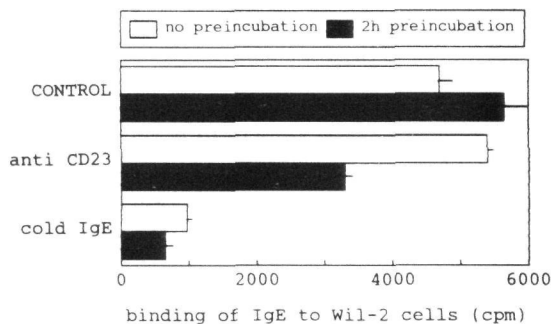


Fig. 4. Effect of anti-CD23 on ^{125}I -IgE binding to Wil-2WT cells. Wil-2WT cells were preincubated for 2 h at 37°C (closed bars) or not (open bars) with a 100-fold excess of anti-CD23 (BB10) or unlabeled IgE. The mixture was incubated with ^{125}I -IgE for 30 min at 0°C . The radioactivity of ^{125}I -IgE bound to Wil-2WT cells incubated with anti-CD23 was $115.2 \pm 0.7\%$ (no preincubation), $58.2 \pm 0.9\%$ (2 h preincubation) of control.

before addition of ^{125}I -IgE. After incubation at 0°C (ice bath), 3-sets of cell suspensions each 0.05 ml were layered on 0.2 ml of FCS in small plastic tubes (0.4 ml Eppendorf centrifuge microtube). The tubes were then centrifuged for 2 min in a Microcentaur Centrifuge (MSE, London, UK). Tips containing the cell pellet

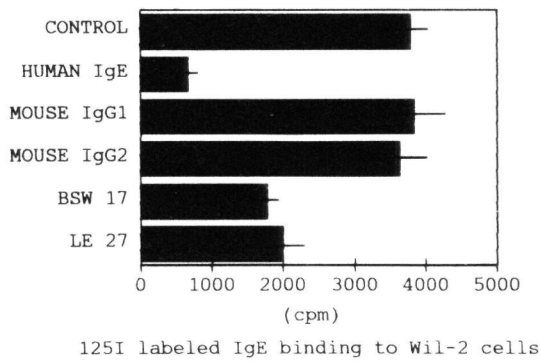


Fig. 5. Effect of murine IgG and monoclonal anti-IgE antibodies on ^{125}I -IgE binding to Wil-2WT cells. Mixtures of ^{125}I -IgE and a 100-fold excess of unlabeled IgE or murine IgG₁ or IgG₂ or monoclonal anti-IgE antibodies (BSW17 and Le27) were incubated with Wil-2WT cell suspensions. The radioactivity bound to Wil-2WT cells was $101.3 \pm 12.3\%$ (mouse IgG₁), $96.0 \pm 7.2\%$ (mouse IgG₂), $96.0 \pm 7.2\%$ (mouse IgG₂), $46.7 \pm 3.4\%$ (BSW17), $52.8 \pm 7.2\%$ (Le27) of control.

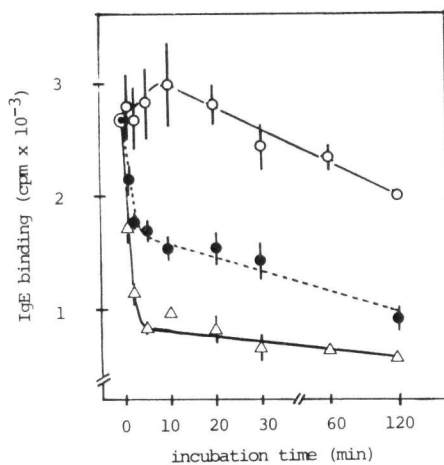


Fig. 6. Effect of monoclonal anti-IgE antibodies on surface bound ^{125}I -IgE. Wil-2WT cells were incubated with a 100-fold excess of Le27 (open circles) or BSW17 (closed circles) or unlabeled IgE (open triangles).

were cut off and their radioactivity was measured in a gamma counter (LKB 1260 multi-gamma counter).

RESULTS

Binding of ^{125}I -IgE to Wil-2WT cells

The specific binding of ^{125}I -IgE to Wil-2WT cells was calculated as total binding minus non-specific binding in the presence of a 100-fold

excess of unlabeled IgE. As shown in Fig. 1, the specific binding of ^{125}I -IgE occurred very quickly, reaching maximal binding after 15 min of incubation. The specificity of IgE binding to Wil-2WT cells was evaluated by competition with other classes of human immunoglobulins. Instead of unlabeled IgE, a 100-fold excess of human IgG₁-lambda, IgG₂-kappa, IgG₃-kappa, IgG₄-lambda, IgA₁-lambda, IgA₂-lambda and IgM-kappa were used for incubation with cells suspensions before addition of ^{125}I -IgE. As shown in Fig. 2, only unlabeled IgE produced competitive inhibition for ^{125}I -IgE binding. The radioactivity bound to Wil-2WT cells was $110.8 \pm 7.2\%$ (IgG₁-lambda), $9.1 \pm 4.0\%$ (IgG₂-kappa), $100.8 \pm 0.8\%$ (IgG₃-kappa), $101.7 \pm 9.4\%$ (IgG₄-lambda), $96.8 \pm 5.1\%$ (IgA₁-lambda), $109.6 \pm 5.1\%$ (IgA₂-lambda), $104.1 \pm 12.9\%$ (IgM-kappa1), $101.5 \pm 7.1\%$ (IgM-kappa2) of control.

As the binding of ^{125}I -IgE to Wil-2WT cells reached a plateau after 10–15 min of incubation, we investigated whether ^{125}I -IgE might be dissociated from Wil-2WT cells. Fig. 3 shows that a 100-fold excess of unlabeled IgE was able to replace cell bound IgE. The immunological specificity for the binding site of IgE was confirmed by an inhibition experiment (Fig. 4) using an anti FcεRII antibody. BB10 a monoclonal anti-FcεRII antibody significantly inhibited IgE binding if this antibody was added 2 h prior to IgE. If IgE and anti-FcεRII were added simultaneously, no inhibition occurred. Thus our data show that IgE specifically binds to Wil-2WT cells with low affinity and that the cell bound IgE can be effectively and rapidly dissociated from the cell surface.

Influence of monoclonal anti-IgE antibodies on ^{125}I -IgE binding to Wil-2WT cells

Next, we investigated whether the above described assay might be used to study the potential role of anti-IgE antibodies in modulating FcεRII binding of IgE. As a model we used murine monoclonal anti-IgE antibodies (BSW17, Le27) and FcεRII positive Wil-2WT cells. Wil-2WT cell suspensions were added to ^{125}I -IgE in the presence of a 100-fold excess of

monoclonal anti-IgE antibodies. Then cells and monoclonal IgG anti-IgE/IgE complexes were incubated for 30 min at 0°C. For control, complete medium was added instead of monoclonal anti-IgE antibodies. Fig. 5 shows that both monoclonal anti-IgE antibodies significantly inhibited the binding of ^{125}I -IgE to Wil-2WT cells. The radioactivity in the cell pellets was reduced to $46.7 \pm 3.4\%$ (BSW17), $52.8 \pm 7.2\%$ (Le27) of control. Both monoclonal murine anti-IgE antibodies belong to the IgG₁ subclass. Therefore we examined whether the murine IgG₁ may nonspecifically inhibit binding of ^{125}I -IgE to cells. Fig. 5 shows that a 100-fold excess of murine IgG₁ (anti-CD8) and IgG₂ (anti-CD3) did not influence ^{125}I -IgE binding. Thus, these two monoclonal antibodies which recognize IgE in the D_{e1} and D_{e2} region are capable of preventing IgE binding to FcεRII.

We then investigated the influence of BSW17 and Le27 monoclonal anti-IgE antibodies on cell bound IgE. After a 15-min incubation of Wil-2WT cells with ^{125}I -IgE at 0°C, a 100-fold excess of monoclonal murine anti-IgE antibodies were added and further incubated at 0°C. As shown in Fig. 6, radioactivity in the cell pellets decreased following the addition of an excess of monoclonal anti-IgE antibody BSW17. However, the monoclonal anti-IgE antibody Le27 did not immediately remove IgE bound to Wil-2WT cells and only a marginal decrease in cell associated IgE was observed after incubation times longer than 30 min.

DISCUSSION

The data presented in this paper reproduce known properties of the low affinity receptor for IgE on a human B cell line (3, 15). We also show that anti-IgE antibodies may interfere with IgE binding to the FcεRII. Since monoclonal murine anti-IgE antibodies are capable of inhibiting ^{125}I -IgE binding to FcεRII and dissociating ^{125}I -IgE from FcεRII on Wil-2WT cells. The effective monoclonal anti-IgE antibody, BSW17 has a specificity for the human D_{e1} region of Fcε. It may be additionally significant that this antibody does not trigger histamine release from human leukocytes (10). On

the other hand, the monoclonal antibody Le27 is specific for the human D_{e2} region and this antibody is capable of triggering histamine release from human leukocytes (10). BSW17, which does not trigger histamine release, is much more effective in dissociating FcεRII bound IgE than Le27. Furthermore, both monoclonal anti-IgE antibodies (BSW17 and Le27) inhibited the binding of IgE to FcεRII. This and their capacity to dissociate surface bound IgE might suggest such a role of naturally occurring anti-IgE antibodies in human sera. Naturally occurring anti-IgE antibodies may contain several kinds of antibodies against diverse epitopes of Fcε in addition to anti-idiotypic antibodies. We have evidence that there might even be antibodies in sera against the same or similar epitopes of Fcε which are recognized by either BSW17 or Le27 (18). It has been reported that naturally occurring anti-IgE antibodies were mainly present in sera as immune complexes (16, 21). The monoclonal antibody against FcεRII (BB10) did only inhibit binding of IgE if added to the cells beforehand, which might be due to the affinity of BB10. At present, we do not know whether our monoclonal anti-IgE antibodies would also interfere with the binding of IgE to the FcεRI present on basophils and mast cells (2, 9). Nevertheless, we believe that our investigations using Wil-2WT cells and murine monoclonal anti-IgE antibodies might be a useful experimental model to study the significance and physiological or pathophysiological role of naturally occurring anti-IgE autoantibody *in vivo*.

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