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Role of immune complexes in atopic dermatitis

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Immune complex formation is a normal event in the course of immune responses, leading to antigen neutralization and elimination (1). Complement activation, binding to cells and tissue deposition are largely dependent on the antibody component of the complex, so the isotypic patterns of response are determinant for the biological activities of complexes. Other factors affecting their role are the nature of the antigen(s), the clearance rate by the reticuloendothelial system, and the size (Ag:Ab ratio) of the complexes.

Low levels of immune complexes are present in all normal individuals for different reasons, but they are subjected to a rapid clearance and do not generally cause clinical symptoms. The development of techniques to identify the antigen within the complexes represents a further step forward, enabling us to distinguish between antigen-antibody complexes and aggregates formed by interactions of antibodies with antiglobulins, antiF(ab')2, anti-idiotypes, and so on.

It is currently accepted that high levels of circulating immune complexes may be considered pathological and cause immunological injury. In human diseases, however, the exact relationship of immune complexes to the nature of the lesion is not yet completely understood, since raised levels of complexes have been found in several different conditions.

The role of common environmental allergens (house dust mites, foods, chemicals, pollens) is still controversial in AD, as is the relevance of immunopathogenical mechanisms so far detected (types I, III and IV, according to Gell and Coombs' definition). Evidence from some studies favors the view that ingested antigens may play a role in the development of AD in predisposed infants, but in young adults with chronic AD, delayed-type hypersensitivity to mites (contact allergy) seems to be a common finding. This heterogeneity of responses to allergens brings our attention to the clinical differences observed in patients, and the possible distinction of different patterns within AD – which should be regarded, not as a clinical entity, but as a syndrome with a spectrum ranging from minor transient manifestations in early childhood to severe, chronic symptoms accompanied by primary or secondary immune dysfunctions at a later age.

Food antigen-antibody complexes in AD

The absorption of intact food antigens across the mucosa into the circulation triggers their combination with serum antibodies. The type and amount of complexes formed differ according to the extent of gut permeability and the spectrum of antibodies present. In normal adults and children subjected to oral challenge with cow's milk, we observed the formation of circulating complexes containing beta-lactoglobulin (BLG) 1–3 h after milk ingestion. The complexes were of IgA and IgG types and their clearance was rapid (2). In both children and adult patients with AD or symptoms related to food allergy, we found the occurrence of higher levels of circulating complexes after reaction to a food challenge (3). By an immune complex-splitting assay (4), we detected food antigens as constituents of the complexes; however, the amount of complexed antigens was similar to that measured in healthy subjects. Major differences were observed when we analyzed the immunoglobulin isotypes involved in immune complex formation, since IgE and IgG predominated (5). Up to 20% of all serum IgE was in

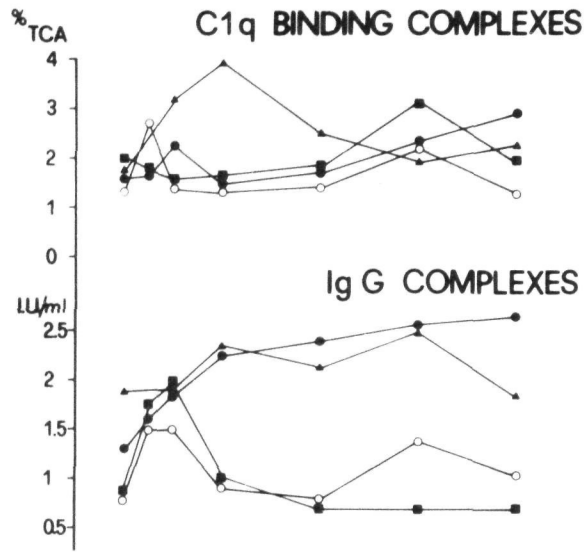

Fig. 1. C1q binding complexes and IgG-containing complexes in AD patients after oral challenge. Results expressed as % maximum radiolabelled C1q precipitable, and IU of IgG precipitable in 2.5% PEG.

complexed form and the complexes were heterogeneous, as confirmed by ultracentrifugation.

In normal individuals, we estimated that a maximum of 0.1% of the antigenic load administered orally could be identified in the circulation by a two-site solid phase radioimmunoassay, which would detect only antigenically intact proteins (6).

Atopic dermatitis caused by food allergy has been described in fully breast-fed infants. This observation may be explained by sensitization *in utero* or, more probably, by intolerance to some component in the mother's milk (7). We found that breast-fed infants with AD had increased levels of IgG antibodies to BLG.

We recently studied a cohort of fully breast-fed infants in cooperation with Dr. Cavagni (Pharma) for presence of AD responsive to cow's milk or egg withdrawal from the maternal diet. BLG was detectable in mothers' milk after drinking cow's milk, but not when on avoidance diet, and this correlated with presence of symptoms in the babies. IgG or IgE antibodies to BLG or other cow's milk proteins both in mothers and babies did not correlate with development of symptoms, so this possibly indicated that other pathogenic mechanisms were responsible.

A primary defect of antigen exclusion mechanisms, including secretory IgA, has been postulated to underlie the development of atopy (8). In AD patients, a primary abnormality of intestinal permeability has also been observed. Using uncharged polymers of low molecular weight, an increased entry has been detected in such patients (9). We investigated the absorption of macromolecular antigens after specific challenge in food-allergic AD patients and found that peak levels of circulating BLG were more than twice those obtained in normal controls (3, 4, 5).

Complexes containing IgE in AD

It would be tempting to assume that antigen-antibody complexes are of critical importance in atopy and particularly in food allergy. Immune complexes containing IgE, detected by various methods, might play a role.

In situ formation of immune complexes has not been studied extensively in human allergic conditions but its occurrence at the intestinal level might result in local inflammation, with increased permeability to food antigens (10). In this context, the presence of systemically circulating complexes after food challenge might represent an epiphenomenon without clinical relevance. This is probably the case in normals, but immune complexes containing IgE have the potential for triggering mediator release from various cell types. In this respect, type I, type III, and other reactions would coexist since each component of the complex (antigen, IgE, other antibodies and autoantibodies) might contribute to the inflammatory event and the development of symptoms. Our knowledge of complexed IgE in atopy suggests that their continuous presence (e.g., caused by interaction with anti-IgE) might contribute to the chronic course of certain diseases. Among these, AD, Hyper-IgE Syndrome and food allergy are pre-eminent although IgE and IgG complexes have also been found in perennial rhinitis and asthma (11). The evidence for the occurrence of complexed IgE in atopy rests mainly on the development of IgE of high mw, or PEG precipitable IgE in food-allergic subjects after challenge, or in asthmatics with sta-

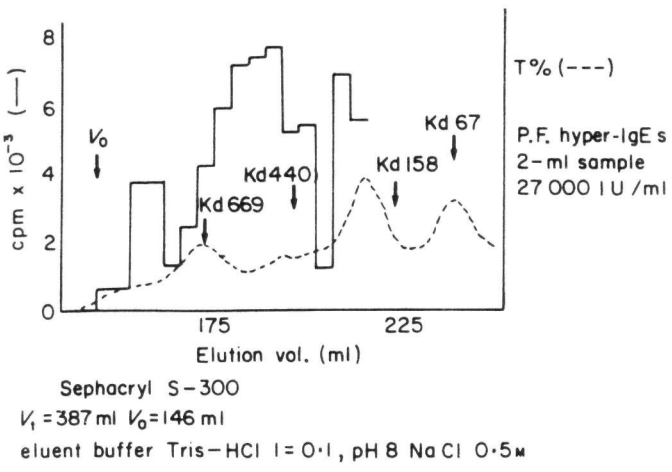

Fig. 2. Heterogeneity of IgE complexes in serum of a case of Hyper-IgE Syndrome. Reproduced with permission of the publisher from ref. 16.

ble disease irrespective of the adoption of hyposensitizing treatment. However, we observed the presence of 2.5% PEG-precipitable IgE and IgG also in patients with food allergy and AD, prior to specific oral challenge, with only minor changes in the samples collected over 6 h (5).

Nawata and coworkers found the presence of IgE and IgG anti-IgE in two discrete peaks in asthmatic patients (12) and in patients with AD (13). In the latter study, they estimated that most, if not all anti-IgE, was in complexed form, with an average mw of about 600 kD, whereas IgE was both free and complexed.

Swainson et al. (14) investigated IgE complexes in AD, and found that 27% of patients lacked high mw IgE by HPLC, but over half showed > 5% of their IgE at an apparent mw of 470 kD. IgG anti-IgE were present in high mw fractions in 2/3 of the sera. In another study, Quinti et al. (15) observed that only IgG anti-IgE, but not IgM, were in high mw form, and the specificity of such autoantibodies was definitely confirmed. IgG anti-IgE were present as monomer, and as part of the immune complexes, of intermediate size in all atopic diseases studied, but large complexes > 19S were found only in AD and Hyper-IgE Syndrome.

We confirmed that, in Hyper-IgE Syndrome patients, IgE complexes were present with mw ranges from 450 to > 1000 kD (16). Within the complexes, which also reacted for iC3b and IgG, we detected food antigen-specific IgE and IgG, and IgG anti-IgE and anti-F(ab')2 activities.

A possible cause for the differences in amounts of complexed IgE and their mw reported in these and other studies may be due to artefacts in the assay measuring IgE.

Using a solid phase noncompetitive assay (Phadebas PRIST®, Pharmacia, Sweden), we observed an inhibition of IgE detection reaching up to 80–90% with a ratio of isolated IgG anti-IgE of 2:1 to 10:1 (17). So reported correlation, or lack of correlation, of IgE levels to IgG anti-IgE values seems to have little importance, since the two activities may interfere with each other in the measuring test.

Effects of IgE complexes

Widely quoted effects of *in vitro* formed IgE complexes with heterologous anti-IgE include the release of arachidonic acid from mouse macrophages (20), inhibition of human neutrophil chemotaxis (21) and activation of human suppressor T cells for IgE synthesis by a myelomatous cell line (22). All these observations may relate to effects of IgE complexes formed *in vivo*, and particularly to those containing auto-anti-IgE. We observed that serum from a patient with Hyper-IgE Syndrome, containing high levels of IgE complexes was not by itself capable of inducing histamine, prostaglandin D2 nor leukotriene release from human lung fragments *in vitro* (18). However, it could sensitize tissue mast cells, so that a monoclonal anti-IgE released these mediators. We also observed that an IgG anti-IgE purified from the serum of an atopic dermatitis patient induced IgE-dependent basophil degranulation with mediator release (19). All attention so far has focused on direct (degranulating) action of IgG anti-IgE or their complexes with IgE on mast cells or basophils. This is clearly due to the prominence of these cell types in allergic reactions and to the presence on their surface of high affinity receptors for IgE (FcER-I). However, they are not the only known receptors for IgE, since platelets, eosinophils, granulocytes, macrophages, Langerhans' cells, B lympho-

cytes, EBV-transformed B cell lines, activated T cells and some T cell hybridomas can bind IgE (for review, see 23). In most cell types, the receptors are of low affinity and in human B cells the low affinity FcER-II has been assigned the cluster denomination CD23, identified by several epitope-specific monoclonal antibodies. Recently, it has been demonstrated that the receptors on B cells are expressed only at mature stages, but before isotype switch (24). Secretory forms of CD23 have been identified (25) in cell lines supernatants and in sera from atopic patients, also bound to circulating IgE. They would give rise to IgE complexes of about 220 kD, but if dimerization occurs, also of 450 kD. We found that fractions of this approximate mw both contain IgE and react with the 3-5 anti-CD23 monoclonal antibody (kindly given by Dr. H. Kikutani, Osaka, Japan).

CD23 is inducible on human B cells by interleukin 4 (26) but not by IgE (24). IL4 (both in mice and humans) exerts important actions on the antibody response, increasing IgE production and IgG_1 (in mice) or IgG_4 (in humans) in atopic or parasitic diseases (27). Modulation of CD23 on B lymphocytes is under influence of cytokines: IL4, which promotes the coordinate enhancement of CD23 and also class II MHC molecules, which are spatially related (28), and IFN gamma, whose suppressive effect on CD23 expression is parallel to the inhibition of IL4-stimulated IgE synthesis *in vitro* (29). Immune complexes containing IgE antagonize IFN gamma in the induction of class II MHC on peritoneal macrophages (30). Moreover a recent study reports that CD23 and class II MHC are significantly increased by soluble IgE-antigen (DNP-OVA) complexes in mouse spleen B lymphocytes (31), whereas IgE alone has little effect. Complexes with multivalent antigens (i.e. of higher mw) were found to be more effective than smaller complexes - a finding also observed for Fc gamma-R induction by IgG complexes. Monomer IgE seems to block cleavage of FcER and release from hybridoma lines (32) so IgE complexes would effectively stabilize IgE binding to receptors, or stimulate *de novo* CD23 synthesis. The effects of IgE complexes are intriguing: increased MCH class II expression is T cell-independent (31), and CD23 expression does not require accessory cells (33), so it seem unlikely that such complexed IgE may act via stimulation of cytokine(s) production.

The presence of FcER-II on several cell types involved in immune disregulation in AD (B and T activated cells, eosinophils, Langerhans' cells) points to the possible relevance of IgE complexes - a stronger ligand than monomer IgE - in the pathology of this disorder. A link between IgE (type I), complexes made of IgE and other isotypes (type III), induction of cell mediated (type IV) reactions can therefore be traced, and the novel findings linking mast cells to neuroendocrine influences might open new insight into the role of immune complexes in AD.

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