



Review

Historic overview of allergy research in the Netherlands

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ABSTRACT

Research in allergy has a long history in the Netherlands, although the relation with immunology has not always been appreciated. In many aspects Dutch researchers have made major contribution in allergy research. This ranges from the first characterization of house dust mite as an important allergen, the first characterization of human Th2 and Th1 T cell clones, to the development of diagnostic test systems. In this overview Aalberse and Knol have made an overview of the major contributions of Dutch immunologists in allergy.

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1. Introduction. Linking allergology and immunology. The pre-IgE era

The relation between allergology and immunology has had its ups and downs. In the first two decades of the 20th century, when the concept of allergy was established, a relation with immunity and antibodies was generally assumed. This was to a large degree due to the fact that many allergic reactions were a side effect of the therapeutic use of animal antiserum, a condition in which precipitating antibodies were easily demonstrable. This situation changed when Coca and Cooke wrote in 1920 in *The J Immunology* an influential paper on the distinction between anaphylactic allergy and atopic allergy. One of the main points was that in atopic allergy (with hay fever as the prototype) no antibody was involved, but a mysterious heat labile substance, which they named the “atopic reagin”. This created a demarcation between atopic allergy and immunology. It took 45 years before the link between allergy and immunology was reestablished.

For an extensive overview of the early history of Allergy worldwide, with all the references, see [1]. The most prominent Dutch pioneers in the pre-IgE era of allergology were Storm van Leeuwen, Voorhorst and Berrens. Their focus of research (with skin tests as their major tool) was mostly on indoor allergens, i.e. house dust.

Storm van Leeuwen was famous for his studies with allergen-free rooms. In his critical and informative state-of-the-art paper

of 1932 (regrettably written in Dutch) he promotes the 10% rule for ideal allergen extracts: no more than 10% false-negatives, no more than 10% false-positives [2]. It is a sobering thought that many extracts currently in use do not achieve this (seemingly modest, but possibly unrealistic) ideal.

Voorhorst and his coworkers M. and F Spijksma became famous by their identification of the house dust mite. For an historical overview, see [3]. Voorhorst was also much interested in the skin test reactivity in human dander extracts, which he considered to be a prototypic form of auto-allergy, reviewed in [4]. It took many years before another contributor to the skin test reactivity of human skin scales became established: proteins derived from a lipophilic yeast currently usually referred to as the *Malassezia* family [5,6]. Because IgE to this yeast is almost exclusively found in patients with atopic dermatitis, whereas in Voorhorst's studies skin test reactivity to human dander is often also found in atopic patients without dermatitis, it is still open whether this skin test reactivity is partially due to auto-allergy. This issue is also relevant in relation to the IgE-dependent histamine-releasing factor, discussed below.

Berrens, a chemist by training, had a very different view on allergens. Based on his work with Bleumink on the skin test reactivity of the cow's milk protein beta-lactoglobulin (published in 1966 in *Nature* [7]) he proposed that allergenicity of all allergenic proteins was due to a modification of the protein structure by a non-enzymatic glycation reaction known as the Maillard-reaction. According to the Berrens hypothesis “the house dust allergen” was allergologically indistinguishable from the cat allergen or any other allergen. The relevance of mites was a matter of fierce discussions between Berrens and Voorhorst. The achievements of Storm van Leeuwen and Voorhorst have been described elsewhere

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[1,3,8]. History has been less kind to Berrens. This was mainly because of his innate tendency to attack scientific orthodoxy and the urge to formulate highly original alternative views in an eloquent and scientific, but also dismissive to anyone unconvinced, way. Unfortunately, the Maillard hypothesis became untenable upon the appearance of monoclonal anti-allergen antibodies and recombinant allergens. There is still a possibility that the Maillard-modification (now known as Advanced Glycation Endproducts, AGE, with a corresponding family of receptor proteins called RAGEs) may be a relevant factor of allergenicity, but this is unlikely to be a major contributor in the initiation phase and is certainly not required for the elicitation phase. Berrens also postulated a unique mode of complement activation by atopic allergens, with a potent house dust fraction as the prototypic complement activator [9–12]. One hallmark of this process was that only human (but not guinea pig) complement was active. This house dust effect was confirmed by Van der Zee [13], but was found to be due to soluble polysaccharides from house dust rather than by allergenic proteins. This property was shared with soluble bacterial polysaccharides, with IgM as an important factor [14]. A recent study in food allergy indicated that complement plays a role in the binding of allergen on B cells via IgG [15] indicating that the role of complement in allergy might be more widely implicated than anticipated before.

With the discovery of IgE in 1966 by Ishizaka and by Johansson, and the development of the RadioAllergo Sorbent Test (RAST) by Wide, Bennich and Johansson in 1967, (for references see [1]), tools became available to investigate the allergenicity of house dust from a different perspective. Using sera selectively positive for mites or cats and performing inhibition of IgE binding to house dust extract by cat and mite extracts, it was found that both mite and cat allergens were found in all Dutch dust extracts, albeit in different ratios [16]. Neither Voorhorst nor Berrens accepted these results as relevant for clinical allergy. Berrens because at that time (early seventies) he did not accept that IgE was relevant, Voorhorst because he did not accept the finding that cat allergens were found in almost all dust samples, including in dust from houses without cats.

Even so, thanks to the discovery of IgE and the availability of IgE tests, the link between allergology and immunology was re-established. But not always whole-heartedly, as is expressed well by the guest editorial on “Allergy and Immunology: wedding of love or marriage of convenience? (A modern medical tale)” [17].

2. Allergen characterization

The Dutch contribution to the history of allergen biochemistry was initially based primarily on RAST inhibition. This was a spin-off of the RAST, which was developed as a very efficient diagnostic test by Wide, Bennich and Johansson in Uppsala in 1967. After a very hospitable and helpful visit in 1970 to Gunnar Johansson in Uppsala, Aalberse introduced the test in Amsterdam at the Central Laboratory of the Blood Transfusion Service CLB (now Sanquin). The test required affinity purified anti-IgE for labeling with ^{125}I , and thus a source of purified IgE. In the absence of a Dutch source of monoclonal IgE, polyclonal IgE was purified (by affinity chromatography) from several liters of plasma from 2 helminth-infested patients [18]. The RAST was performed with a slight modification of the Uppsala protocol: the allergen was coated to CNBr-activated Sepharose-beads in suspension, rather than to cellulose particles or paper discs. This had two advantages. Firstly the high binding capacity of the Sepharose beads (typically 5 μg protein per test) allowed the testing of crude allergen extracts, including house dust extract. Secondly, the suspension could be titrated to optimize the reaction conditions for use in RAST inhibition.

The obvious disadvantage of the use of a suspension of beads was the need to centrifuge the test tubes (nine times). This manual

procedure might seem to be incompatible with high throughput testing, but the diagnostic department at the CLB was able to run well over a 1000 test/day. This large-scale diagnostic testing resulted in a statistical analysis of 150 000 sera tested in the period 1983–1990 on a panel of five or six (for children) allergens, showing a small, but statistically significant “horoscope” effect, not only for seasonal allergens but also for perennial allergens, with sensitization to cat, dog, egg and milk being more prevalent in patients born in wintertime (possibly reflecting a protective role of vitamins A and/or D in the first half year of life, or an unfavorable role during the second half year of life) [19]. Of even more interest was the availability of a wide range of sera with interesting IgE specificities. As an alternative to the bead suspension technology a macro-bead procedure was developed in 1986 [20]. It involves the prior modification of the allergen with a hapten (or biotin). The serum is incubated with the hapten-conjugated allergen in fluid phase. The allergen (with or without antibody attached) is next extracted by added a single macro-bead (7 mm diameter polystyrene) coated with anti-hapten antibody (or streptavidin). IgE bound to the bead is detected as usual with labeled anti-IgE. Presumably because of the fluid-phase conditions, this technology was found to give much more sensitive and accurate RAST-inhibition profiles.

The allergenic activities in house dust offered interesting scientific challenges that were studied with the combined use of RAST and RAST inhibition. This resulted in the identification of food remnants, such as egg and milk proteins, as neglected contributors, even if it is still unclear how much of these settled allergens will become airborne in domestic situations [21]. A similar argument holds for allergenic activity derived from invertebrates other than mites. The common source of house dust is the content of a vacuum cleaner bag. Dead insects contain IgE-reactive substances, much of which is due to muscle proteins such as tropomyosin [22]. It is obviously relevant to take into account that these muscle proteins are often cross-reactive with IgE induced by the consumption of shrimp [23]. IgE reactivity to house dust extract is occasionally idiosyncratic, reacting preferentially with house dust of the IgE donor. The specificity of IgE of one such patient (Ka), has so far remained a mystery, despite testing hundreds of allergen sources materials and despite the help of intrigued colleagues all over the world. Observations like this indicate the ongoing need to be able to perform autologous house dust RASTs [24].

For the characterization of allergens in other source materials, the traditional physicochemical approach has been used successfully for peanut allergens by Koppelman et al. [25–27]. Even if recombinant technologies have largely replaced protein-based characterization, the need to keep a close watch on the structure of allergens and their post-translational modifications as obtained from their natural sources is illustrated by several Dutch peanut studies [28–30].

As mentioned in the introduction, Voorhorst was much interested in the skin test reactivity of extracts of human dander. This activity suggested the presence of an autoallergen, even if the presence of *Malassezia* derived allergens in these extracts is a complicating factor. Another potentially relevant factor was described by Susan MacDonald in 1987: the IgE-dependent Histamine-Releasing Factor (HRF) [31]. This macrophage-derived factor was described as (1) inducing basophil degranulation in an IgE-dependent way, (2) being distinct from classical allergens and (3) discriminating between two types of IgE, called IgE⁺ and IgE[−]. A protein with some of these activities was purified, sequenced and identified as a protein known as Translationally Controlled Tumor Protein (TCTP) [32]. Studies by Pasmans [33,34] and Kleine Budde [35] confirmed the presence of IgE-dependent HRF activity of supernatants of activated monocytes, but indicated that these supernatants contain in addition variable amounts of the chemokine MCP-1, which has a potent basophil-priming activity

[36]. TCTP similarly has been described to have a basophil- and eosinophil-priming activity. The conclusion from the Amsterdam studies is that monocyte-derived HRF activity is presumably a combination of the activity of non-IgE dependent priming factors and IgE-dependent autoallergens [37]. None of the IgE auto-allergens detectable by immunoblotting techniques could explain the IgE-dependent reactivity in the monocyte supernatants [38]. Even with the Sepharose-based RAST technology no convincing IgE binding was demonstrable in a semi-purified monocyte supernatant. This might indicate that the supernatant contains the auto-allergen at the ppm level, which would be enough to activate IgE-sensitized basophils, but not enough to achieve a positive RAST (or immunoblot).

2.1. Monoclonal antibodies to allergens

A major breakthrough for allergen studies was the development of the monoclonal antibody technology. The first monoclonal antibodies to an allergen (the cat allergen Fel d 1) were reported at the Paul Ehrlich meeting in 1981 [39]. At this meeting the preferred use of monoclonal antibodies for analytical purposes was a hybrid assay in which the monoclonal antibody was used to capture the allergen, which was then detected with IgE (i.e. serum from an allergic patient) and labeled anti-IgE. This is a dual-purpose assay, useful both for the measurement of IgE specific for this allergen as well as for the measurement of the allergen.

During his sabbatical in 1986 in the lab of Tom Platts Mills and Martin Chapman in Charlottesville, VA, Aalberse convinced his hosts that monoclonal antibodies were not only applicable for analytical purposes, but could also be used to efficiently purify allergens. The showcase was Fel d 1 purified from house dust [40,41]. This material was used to prepare a panel of additional anti-Fel d 1 antibodies. The affinity-purified allergen was subsequently sequenced and cloned by Jay Morgenstern working at ImmuLogic in Cambridge, MA [42]. Back in Amsterdam, monoclonal antibodies to Can f 1 and Can f 2, two dog allergens, were similarly used to purify the proteins and ship these to ImmuLogic for sequencing, cloning and expressing these allergens [43,44]. Monoclonal antibodies to two other major allergens that were produced in Amsterdam were instrumental in defining their structure and relevance. One antibody proved to be directed to an important but elusive 14 kD mite allergen, now known as Der p 2 [45]. Another antibody was directed to an IgE-binding protein from grass pollen with a molecular mass very close to the well-known grass group 1 allergen [46]. This allergen is now known as grass group 5 allergen, which has been found to have an allergological relevance similar to the grass group 1 allergen.

Monoclonal antibodies to allergens are usually highly specific (sometimes actually more specific than desirable, by distinguishing between allergens that are usually indistinguishable for human IgE antibodies [47], but sometimes a monoclonal antibody can point toward unexpected and clinically relevant cross-reactivity. An example is an antibody 1A6, obtained from mice immunized with a house dust mite extract [23]. This anti-tropomyosin antibody was found to react not only with mites, but also with shellfish, and a wide variety of invertebrates, including cockroach, silverfish and nematodes.

2.2. Allergenicity and allergen cross-reactivity

Allergen structure and its relation to allergenicity and allergen cross-reactivity have been discussed in several reviews [48,49]. One point of debate is to what extent allergens differ from “non-allergens”. Aalberse argued in favor of a relatively unrestricted view of allergenicity: almost all antigens that can stimulate B cells in an antigen-specific manner can in principle induce IgE

antibodies. Nevertheless, upon natural exposure, some antigens are more likely than others to induce IgE, sometimes because of some biological effect of the antigen itself or associated components, but mostly because of differences in exposure levels, barrier effects, etc. [48,49].

Cross-reactivity among related allergens is a common problem in allergology, as it has an impact on test specificity as well as treatment. Some of these cross-reactivities are not immediately obvious, but phylogenetically plausible (such as apple allergy in birch-pollen sensitized patients), but others come as a real surprise. This is true for some of the cross-reactions due to tropomyosin mentioned above. It is even more so for cross-reactivities due to glycosylation. The best-studied example is the Cross-reactive Carbohydrate Determinant (CCD), described in 1981 [50]. It is an N-linked glycan structure found in a wide variety of plant- and invertebrate glycoproteins, but not in mammals [51]. Its absence in mammals and its presence on some known allergens, notably in hymenoptera venoms, make it a textbook hapten-carrier system. Still, it took many years before the concept was generally accepted. One source of confusion was the vague notion that it all looked like a lectin-type interaction, and IgE is highly glycosylated. Such lectin-type interactions of IgE are well known, for example the mannoside-susceptible interaction of IgE with pea extract, in which the pea lectin interacts with some of the glycans on IgE. In contrast, IgE to CCD itself behaves like a lectin by interacting with glycans in allergen source materials. One major step in the acceptance of the CCD concept was the introduction of CCD reagents as test components in commercial diagnostic kits such as the ImmunoCAP. This allowed clinicians to see the IgE reactivity to CCD in their own patients and to appreciate their confusing effects on the specificity of IgE assays. One striking feature of the IgE–CCD interaction is that it has a very low tendency to trigger effector cells [52]. In other words: it is a major confounding factor. The jury is still out on the proposal that CCD is occasionally a relevant (=symptom-inducing) epitope, perhaps in combination with IgE antibodies to the carrier protein.

We now know that many cross-reactive allergens exist. As early as in 1987 Calkhoven et al. showed that the cross-reactivity among birch pollen, vegetables and fruits was due to at least three specificities: PR-10 proteins related to the major birch protein Bet v 1, CCD, and a 14 kD protein [53] subsequently identified by Valenta et al. as profiling [54]. The same holds true for cross-reactivity among invertebrates, which involves other proteins in addition to CCD and tropomyosin [55].

2.3. Recombinant allergens and their use in allergen standardization

Van Ree and his coworkers have been internationally active in improving the reagents and procedures for the standardization of allergens. In addition to developing some of these reagents, notably natural and recombinant proteins such as Lipid Transfer Proteins [56] and oleosins [57] and hazelnut 2S allergen [58], but also monoclonal anti-allergen antibodies, he was/is heavily involved in large international collaborative projects such as CRE-ATE [59] and EuroPrevall [60]. EuroPrevall is an international consortium (with Ronald van Ree as the Dutch representative) responsible for the European Community Respiratory Health Survey on the prevalence, cost and basis of food allergy across Europe (http://cordis.europa.eu/result/rcn/51771_en.html).

3. Antibodies: IgE and IgG4

The first signs of an involvement of IgG4, in addition to IgE in allergy date back to the 2nd International Congress of Immunology in Brighton (1974), where three groups independently presented

data on this topic: Cambridge (Devey [61]), Birmingham (Stanworth) and Amsterdam (Van der Giessen and Aalberse [62]). Both pro- and anti-allergic effects of IgG4 were considered. The pro-allergic effects initially received most attention, particularly based on the studies by Parish on “short-term sensitizing IgG antibodies” IgG-STs. Some doubts on this pro-allergic effect of IgG4 were raised by the studies by Van Toorenbergen et al. showing that IgG4 did not sensitize basophils for allergen-induced histamine release and that IgG4 antibodies were found in a wide variety of non-symptomatic subjects [63–65]. Studies in beekeepers showed that IgG4 antibodies were preferentially induced upon persistent antigen exposure, without causing any allergic problems [66]. The studies in allergic subjects receiving allergen-specific immunotherapy (consisting of multiple injections of increasing doses of allergen extract) suggested a relation between IgG4 and blocking (=allergen-neutralizing) antibodies [67]. An anti-inflammatory effect of IgG4 was also suggested by the observation that IgG4 antibodies interfered with immune-complex induced complement activation [68]. IgG4 was found to be markedly enhanced in helminth infections [69,70].

During these studies in beekeepers and immunotherapy patients it was noted that IgG4 antibodies behaved as monovalent, non-precipitating antibodies [71]. Recombinant chimeric IgE, IgG1 and IgG4 antibodies to allergens were introduced in 1997 by Schurman et al. [72]. These antibodies have been found to be important for the standardization of assays of anti-allergen antibody [72]. Additionally, they proved valuable tools for the investigation of biological and physicochemical properties of the immunoglobulin isotypes. One example is the potential stimulating effect of IgG antibodies on allergen-induced IgE-dependent basophil activation [73]. The availability of recombinant IgG4 antibodies was expected to provide clues about the structural basis of the apparent monovalency. The initial hypothesis was a steric interference between Fabs following antigen-binding by one of the Fabs. However, the chimeric IgG4 antibodies were found to be bivalent [74]. This observation, combined with the reported lability of the disulphide linkage of the heavy chains of IgG4, led to an investigation of an alternative hypothesis: an exchange of IgG4 half-molecules (later referred to as “Fab-arm exchange”) after secretion by the plasma cell. Support for this hypothesis was obtained by *ex vivo* experiments with serum from subjects with high levels of IgG4 antibodies to two distinct antigens (for example to the mite allergen Der p 1 and the grass pollen allergen Dac g 5). In such sera bi-specific IgG4 antibodies were found (i.e. IgG4 antibodies cross-linking these two different antigens). The level of these bi-specific antibodies closely corresponded to the levels expected if IgG4 was fully scrambled. When these results were initially reported (after a long struggle with reviewers) they were met with overwhelming skepticism. Many experiments later, including passive transfer of mixtures of recombinant IgG4 antibodies into mice (because simple mixing of IgG4 antibodies *in vitro* did not result in measurable levels of Fab-arm exchange) the evidence was sufficiently strong to convince most critics, including reviewers of *Science* [75]. One of the unexpected newer findings was that the anomalous structural behavior of IgG4 was not only due to the hinge abnormality, but was also strongly influenced by its weak interaction between the CH3 domains [75–79]. The structural basis of the altered CH3 stability of IgG4 compared to human IgG1 was established by solving the structure of the IgG4 Fc [80] and the CH3 dimer [81]. These findings helped to solve another unusual feature of IgG4: it behaves like an IgG rheumatoid factor [82–84]. This was found to require both the hinge lability and the instability of the CH₃–CH₃ interaction of IgG4 [78].

Non-precipitating antibodies have been known for a long time. An interesting phenomenon was proposed as a cause of monovalency by Margni et al.: asymmetric Fab glycosylation demonstrable

by affinity chromatography using mannose-specific lectin Concanavalin A [85]. In hindsight, it fits nicely with the Fab-arm exchanging property of IgG4 that the asymmetrically glycosylated immunoglobulin fraction was predominantly IgG4. Intriguingly, Kosthe et al. [86] showed that IgG4 binding to a mannose-specific lectin from banana was an exceptionally high, and much higher than to binding by IgG1. Because part of the binding of the lectin to IgG4 was inhibited by mannoside, this supports the notion that mannose-rich Fab glycans are relatively common on IgG4, presumably more than the twofold increase that could be ascribed to Fab-arm exchange. This could be relevant for the aberrant IgG4 B cell expansion/differentiation found in the hyper-IgG4 syndrome (discussed below). It is, however, still a puzzle why a substantial fraction of the IgG4 binding to the lectin is mannoside-resistant, and thus is presumably due to a glycan-independent interaction.

4. T cell polarization in allergy

In 1986 an important concept in the allergic immune response was developed by Bob Coffman and Tim Mosmann at DNAX, Palo Alto, USA, that mouse T helper cells could be divided in type I and II, based on the cytokine-releasing phenotype. This phenotype was then also confirmed in humans by the group of Martien Kapsenberg, AMC Amsterdam, where Wierenga was the first to publish on human T helper clones specific for house dust mite allergen. The T cell clones from non-atopic individuals produced mainly IFN- γ and no IL-4 which was characteristic for Th1 clones, whereas T cell clones from allergic donors produced IL-4 and no IFN- γ , typically TH2 clones [87]. The collaborative work of Neijens and Savelkoul in Erasmus Medical Center in Rotterdam examined in detail the developing immune system at the level of T cells in the first year of life. There was a clear Th2 polarization in allergic high risk children which was correlated to a poor production of IFN- γ [88]. In asthmatic patients Borger et al. in Groningen described that peripheral blood T cells activated *in vitro* have enhanced mRNA expression of the Th2 cytokines IL-4 and IL-5, of which IL-5 was correlated with diminished lung function and IL-4 with serum IgE [89]. When resting CD4⁺ T cells from atopic dermatitis patients were analyzed by gene array analysis, this typical Th2 profile could not be found, despite the highest serum IgE levels in this group of patients by Hijnen et al. in Utrecht. Expression of mRNA specific for enhanced T cell homing toward skin and genes involved with diminished control of active T cells were demonstrated [90].

In the allergic affected tissue there was also T helper polarization found. Remarkably, this turned out to be more plastic, with Th2 in the acute reactions, but also Th1 in the more chronic allergic inflammatory sites [91]. The long-lived fate of allergen-specific T cells both in blood as in tissue further hints at roles of these cells in acute as well as chronic allergic reactions [92].

The Th1/Th2 concept has expanded with Th9, Th17, Th22 subsets and also so-called regulatory T cells (Tregs) have been described. Interleukin 10 is one of the cytokines released by Tregs and studies by Tiemessen in Utrecht indicated that IL-10, and not so much IFN- γ , was important in tolerance for cow's milk allergen in food allergic individuals [93]. Moreover, Hijnen in the same group described that also CD8/cytotoxic T cells in skin a potent release of cytokines such as IFN- γ and IL-13 [94].

5. The role of dendritic cells in polarizing T cells and in allergic inflammation

Later work in the group of Kapsenberg focused on dendritic cells (DC) controlling the polarization of T cells. Results by Pawel Kalinski and others in this group demonstrated that in addition to presentation of antigenic peptides (signal 1) and costimulation

(signal 2), DC delivered a polarizing signal that determined the Th phenotype (Signal 3) [95]. The dendritic cells also depend on external signals for their polarizing effect. Esther de Jong in the same group described different microbial compounds that differentially can change DC functions in more Th2 or Th1 polarizing factors [96]. It has been known for quite some time that helminth-infected individuals display very high IgE levels and a Th2-polarized phenotype. The research group of Maria Yazdanbakhsh in the Leiden University Medical Center has examined the interplay between helminthes and our immune system. In one of their studies van der Kleij demonstrated that *Schistosoma mansoni* lyso-phosphatidylserine does direct DC polarization in part via activating the Toll-like receptor 2 [97].

In the Erasmus Medical Center the group of Bart Lambrecht develop an additional concept on the role of DC in allergic inflammation. They described the pro- or anti-inflammatory action of local DC subsets in the lung in mouse models of allergic inflammation. The so-called plasmacytoid DC can be considered anti-inflammatory and depletion of this DC subset diminishes the requirements of adjuvants in their allergic mouse models [98]. In addition, depleting the lung CD11c+ DC population strongly inhibited the allergic inflammatory phenotype in the lung [99].

A special subset of DC is so-called Langerhans cells. These are considered to represent a somewhat more immature DC phenotype, because they are well able to uptake antigens, but not yet able to present the processed antigenic peptides. These cells are prominent in the epidermis of skin. Bruijnzeel-Koomen in Utrecht described that in skin of patients with atopic dermatitis these cells carry IgE on their plasma membrane [100]. Later studies demonstrated that this IgE was binding to the FcεRI-α chain, the same structure which was considered before to be expressed on basophils and mast cells. Geert Mudde in the same group demonstrated that IgE on the Langerhans cells made these cells able to pick up 100–1000 lower concentrations of allergens than without IgE on their plasma membrane [101].

6. Fate of IgE-switched B cells

Already in the early 1990s Van der Stoep et al. investigated IgE B cells from three patients with atopic dermatitis, both ex vivo and after in vitro switching [102,103]. In the freshly isolated cells they observed only direct switching, which contrasts with the mouse data suggesting predominance of indirect switching. They investigated mostly the VH5-subset, which was over-represented in their study. They confirmed the virtual impossibility to expand IgE-positive B cells in vitro, which they assumed to be due to the very low expression of CD40 and the low frequency of CD23 expression. They conclude that these cells are at a late stage along the B cell maturation pathway, just before the plasma cell stage. These data are not fully compatible with recent data by Berkowska et al. [104]. Lamers et al. provided a further reason why the IgE-switched B cell has an unusual fate by showing that the membrane form of IgE is poorly produced [105,106]. These data suggest that the low level of IgE production is not only due to low frequency of the IgE class switch, but also by a post-switch expansion/differentiation blockade. Upon analysis of the fine-specificity of IgE to a single allergen (the mite allergen Der p 2) it was concluded that the IgE response to this allergen involves many different clones, which would not be expected if the switch itself was the only road block on the way to IgE production [107]. Based on these and other data, Aalberse and Platts-Mills reviewed in 2004 the origin and fate of the IgE switched B cell in relation to the type of antigen [108]. Conventional antigens like tetanus, with low IgE/IgG ratios (typically <0.01), are proposed to have a different effect on the immune system than classical atopic allergens such as allergens from pollen and mites with IgE/IgG ratios often >0.1. Moreover, it is concluded that

IgE-switched B cells are rare and that these B cells are in general different from traditional B memory cells. Persistence of atopic allergy is mostly due to long-lived plasma cells rather than to B cell memory. With the conventional antigens, a form of B cell memory is available for IgE, via the indirect switching pathway. However, for the atopic allergens this is only an option in tertiary lymphoid tissue, particularly in inflamed airway mucosa [109], which results in relatively short-lived plasma cells. These differences have to be taken into account for the translation of rodent studies to human allergy [110].

Since both IgE and IgG4 responses are dependent on IL-4, it does not come as a surprise that antigens that induce an IgE response (such as atopic allergens, helminth antigens and hymenoptera venoms) are also efficient inducers of IgG4 responses. However, some intriguing differences between IgE and IgG4 responses have been found. One striking difference is the kinetic profile of the antibody response, e.g. to bee venom in a novice beekeeper: very fast for IgE (2 weeks), very slow for IgG4 (>3 months of repeated stings) [111]. Also at the repertoire level (epitopes on a single allergen, which was in these studies the major cat allergen Fel d 1) IgE antibodies were found to differ from IgG4 antibodies [112–114]. Until recently, IgG4-switched B cells were hard to find. This was partly a technical problem: most anti-IgG4 antibodies react with an epitope close to the C-terminus which is partially shielded in the IgG4 membrane form. When a more suitable antibody is used, IgG4 B cells were found in a frequency close to the one expected from the plasma IgG4/IgG1 1:10 ratio [115]. This low frequency refuted a hypothesis suggested earlier [108] that IgG4 levels were lower than IgG1 levels in the early phase of the antibody response to bee venom because of a block in the terminal differentiation of the IgG4-switched B cell. It now looks more likely that the IgG4-switched B cell may have a problem similar to the IgE-switched B cell (perhaps as a side effect of their common switching factors, IL-4). With the availability of the more efficient anti-IgG4 antibody it will now be possible to investigate in more detail the IgG4 response at the B cell level, both in allergic subjects and in hyper-IgG4 patients, such as patients with “autoimmune” pancreatitis and other instances of the spectrum of IgG4-related diseases (IRD) [116]. This hopefully may help us to better understand not only these hyper-IgG4 conditions, but also some of the anomalies of the IgE response.

7. Allergic inflammation

Eosinophilic and basophilic granulocytes are considered important effector cells in allergic inflammation, are increased in the blood of allergic individuals and can be found in allergic affected tissues. De Monchy in Groningen was the first to describe that eosinophils infiltrate the pulmonary tissue after a local allergen challenge during the so-called allergic late phase reaction [117]. Similar findings were later described in nasal tissue by Godthelp for eosinophilic infiltration in nasal tissue [118]. The connection between the lower and upper airways in this respect was later demonstrated by Braunstahl in the group of Fokkens in Rotterdam, who demonstrated that a nasal allergen provocation led to tissue eosinophilia in both upper and lower airways [119]. The same group also identified infiltration of basophils in both nasal and bronchial mucosa following segmental bronchoprovocation [120]. In skin of atopic dermatitis patients also eosinophil infiltration was demonstrated in lesional skin, as well as after induction of a local allergic reaction by application of allergen [121]. In experimental mouse and guinea pig models the role of IL-5 in the eosinophilic infiltration, as well as in the initiation of the allergic inflammation was demonstrated by the group of Van Oosterhout in Utrecht [122,123].

Via the development of methods to purify the more rare eosinophilic and basophilic granulocytes from human blood the group of Dirk Roos in the CLB in Amsterdam described the

functional properties of these cells. It was Yazdanbakhsh et al. in this group that described that eosinophils use both cationic proteins and oxygen radicals to kill parasites [124]. The signal transduction mechanisms underlying the so-called priming, pre-activation, state of eosinophils was examined by Koenderman et al. [125]. Later on Koenderman described while in the University Medical Center Utrecht the increased chemotactic movement of eosinophils of allergic asthmatic patients which might be underlying the rapid tissue infiltration of eosinophils in these patients [126]. Another hot topic in eosinophil research in these days was the hypodense eosinophils, specifically found in blood of allergic individuals. It was Hoekstra et al. in Groningen that proved that this hypodensity was an *in vitro* artifact caused by isolation of leukocytes by dextran [127].

Basophilic granulocytes are the least abundant granulocyte in the blood and therefore a challenge to isolate in sufficient amount of untouched and purified populations, especially before the era of immunomagnetic isolation techniques. Knol in the group of Dirk Roos described differential releasability of the basophils from different individuals as well as distinct differences in signal transduction in these cells [128]. The discovery of the localization of the CD63 in the histamine-containing granules in basophils which was exposed on the plasma membrane after degranulation, opened a new tool via monitoring basophil activation by flow cytometry [129]. This has led to a novel cellular approach in allergy diagnostics named Basophil Activation Test and is increasing being used in routine diagnostics. That the concentration of serum IgE was an important determinant for the level of high affinity IgE receptors on human basophils was already shown in 1977 by Stallman using fluorescence microscopy [130]. Later studies in by Toonenbergen indicated similar mechanisms in rat mast cells, although mast cell expression of high affinity IgE receptors was one day slower than IgE level increase in serum [131].

In allergic inflammation there is also a role of allergens directly activating tissue cells. Most probably are some of the enzymatic activities responsible for this potent innate activity of, at least part of, the allergens. Kauffman in Groningen demonstrated protease dependent epithelial cells activation by fungal allergens. These activated epithelial cells displayed a changed morphology as well as cytokine release [132]. Several enzymatic activities have been described for house dust mite and different extracts of house dust mites are able to activate both human and mouse lung epithelium and seems to be important for the allergic sensitization of mouse models [133].

8. Tolerance in allergy

Allergens are in general considered to be harmless antigens and therefore a typical immune response toward allergens is a tolerant one. Thepen and Holt demonstrated that the alveolar macrophage population in the lung is actively limiting the local immune response toward allergens. The macrophages achieve this by inhibiting the functions of antigen presenting cells [134]. The same Free University Medical Center (VUMC)/Australian collaborations also demonstrated that elimination of the alveolar macrophage population resulted in a marked increase of IgE responses to inhaled allergens [135]. It is without doubt that the gut immune system is the system that needs a strong tolerance network taken in account the huge amounts of foreign antigens in our daily diet as well as the enormous number of intestinal microflora. Van Halteren from the same group showed that the mucosal Th2 response is differentially regulated via the oral route than via the nasal route, most profoundly via differential regulation of IgE and IgG1 in mouse models [136]. Unger showed later that the tolerance induction via the nasal route induced regulatory T cells that regulated the ovalbumin-specific responses [137].

Further insight in tolerance for allergens on an individual level has been provided by the work by the group of Maria Yazdanbakhsh at the Leiden University Medical Center (LUMC). Their work focused on the high IgE levels in helminth-infected individuals in developing countries. Surprisingly although the helminths infected individuals had far greater IgE levels in serum, including allergen-specific IgE, their allergen-induced skin prick test results were decreased compared to non-infected individuals [138]. An important player in this effect is probably the helminth-induced production of IL-10, which is potent immune-suppressive cytokine. Importantly, the inhibitory effect of the helminth infected patients on the skin prick test with allergen was reversed after treatment [139]. The hypothesis on this effect is that in the developing countries the immune system is constantly being stimulated by infectious agents which requires a strong regulatory network to prevent immune hyperactivation, or immune hypersensitivity. In the westernized countries there is less pressure on the immune system by infections, which will lead to a less strong regulatory network which can more rapid result in immune hyperactivation/hypersensitivity finally being a trigger for allergies or autoimmune disorders [140].

That IL-10 is an important mechanism in allergy to maintain tolerance was already discussed before at the level of cow's milk allergen-specific T cells [93] in food allergic patients. In addition to limiting the activity of several immune cells, IL-10 is involved in promoting IgG4 production by B cells. Indeed de Ruijter et al. described in food allergic patients that are tolerant for cow's milk also increased levels of cow's milk specific IgG4 [141]. Krop et al. demonstrated that the role of IgG4 is not clear-cut, because IgG4 levels against rodents did not protect against allergic sensitization in laboratory animal workers [142]. Work in the group of Erika von Mutius in Munich on the mechanisms in the hygiene hypothesis has indicated that the farm environment is beneficial in developing tolerance for allergens. In this a strong factor is the consumption of raw milk in infants and young children. Van Neerven et al. have demonstrated that there are multiple immunomodulating factors in raw milk, including IL-10 and TGF- β which might be underlying this [143]. This might also be one of the mechanisms of the hypothesized protective effects of breast milk in allergen development.

9. Genetics, epidemiology and intervention

The group of Dirkje Postma in Groningen has been examining the genetics in the development of allergy and asthma. Important in their initial work was a cohort of patients from Astmacentrum Beatrixoord in Haren. In the 1990s linkages were described by this group, in collaboration with Deborah Meyers and Eugene Bleeker from Johns Hopkins University in Baltimore at chromosome 5, which region was already highlighted for the regulation of IgE [144]. With better techniques, as well as bigger cohorts, it became possible to specifically look at genes involved and several genes linkages promotor polymorphisms, genes and gene-gene interaction have been described by this group since. For instance, Koppelman et al. demonstrated an association of a promoter polymorphism of the CD14 gene and atopy [145]. In addition, the PCDH1 gene encoding Protocadherin-1, a protein involved in cell-cell boundaries was demonstrated as a susceptibility gene for bronchial hyperresponsiveness [146].

In different epidemiology studies within the Netherlands different aspects of allergy have been examined. Work-related allergic symptoms are of concern both for employers and employees. Some examples of Dutch contributions to the occupational allergy field are the investigations in the laboratory animal workers [147,148], pig farmers [149], the baking industry [150] and the greenhouse workers [151].

Allergen exposure in the home environment has been investigated in many studies. Two intervention studies attracted much attention. The starting point for the PIAMA birth cohort was the investigation of the effect of mite-impermeable covers on mite sensitization [152]. Disappointingly, the effect of the intervention proved too hard to study because of an unusually cold winter season, resulting in low mite allergen levels even without the intervention: La mano de DIOS? [153]. This birth cohort has been a major success otherwise and is still going strong. Another study on the effects of mite-impermeable covers was looking at symptom reduction in already sensitized subjects. A significant but modest (threefold) reduction in allergen levels was found, without a significant effect on symptoms both for allergic rhinitis as atopic dermatitis [154,155]. One limitation for both studies was the dilemma: double-blinding prevents a thorough mite reduction protocol, which would have included removal of carpets, etc.

Another type of allergen exposure that is open to intervention is the neonatal exposure to cow's milk. It had been argued as a major risk for sensitization, but also as a tolerance-promoting preventive option. This was investigated in the double-blinded BOKAAL birth cohort study [156,157]. The intervention had no effect either way. The KOALA birth cohort has collected interesting data on immunizations, infections and gut flora in relation to allergic sensitization [158,159].

10. Concluding remark

Because of constraints in space and time we were unable to highlight in this review all the Dutch contributions to the field. Even so, we hope that it is clear that the impact of Dutch scientists in the allergy field has been substantial in many areas in the field. This was to a large degree due to the generous support of a.o. the Netherlands Asthma Foundation (currently the Lung Foundation Netherlands).

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