Linfócitos e a Reacção Alérgica Cutânea Tardia em Humanos

L. TABORDA BARATA*, S. R. DURHAM**

SUMÁRIO

Estudos imunológicos têm vindo a apontar para o facto de a reacção alérgica tardia induzida por alergenos poder representar um tipo específico de hipersensibilidade retardada na qual linfócitos T e eosinófilos detêm um papel importante. Estudos com a técnica de hibridação *in situ* demonstraram neste tipo de reacção uma sobreexpressão de mRNA para citoquinas do tipo Th2 (IL-4 e IL-5). As propriedades biológicas destas citoquinas incluem a regulação de IgE e a promoção de eosinofilia tecidular. Qual destes fenómenos será mais importante em termos de alergia ainda é um campo a explorar. A cinética de expressão destas citoquinas na reacção alérgica tardia na pele é semelhante à da resposta macroscópica, atingindo o máximo entre as 6 e as 24 horas após contacto com alergeno. A maior parte das citoquinas Th2 parece ter origem em células T. Corticosteróides (na mucosa nasal e brônquica e na pele) inibem a expressão de mRNA para IL-4 e/ou IL-5. Por outro lado, a imunoterapia parece aumentar a produção de citoquinas Th1, particularmente interferão-gama. Uma terapêutica mais orientada e específica destes aspectos celulares referidos (p.e. imunoterapia específica de células T, anticorpos monoclonais anti-IL-5, antagonistas dos receptores para IL-4 e/ou IL-5, receptores solúveis de IL-4 e/ou IL-5 ou mesmo interferão-gama ou IL-12 em administração tópica) poderá ser tão eficaz quanto os próprios corticosteróides e, possivelmente, ter menos efeitos secundários.

* Clinical Research Fellow & Honorary Registrar JNICT grant recipient.
** Senior Lecturer & Honorary Consultant Physician
Dept. Allergy & Clin. Immunology
National Heart & Lung Institute,
Royal Brompton Hospital, London, UK

SUMMARY

LYMPHOCYTES AND THE HUMAN ALLERGEN-INDUCED LATE PHASE RESPONSE

Immunological studies have confirmed that human cutaneous allergen-induced late responses represent a specialized form of cell-mediated hypersensitivity in which T lymphocytes and eosinophils are prominent. ''In situ'' hybridization studies have shown preferential expression of mRNA for so-called "Th2-type" cytokines. The biological properties of these cytokines include IgE regulation and promotion of local tissue eosinophilia. Which is more important (IgE or eosinophils) is unknown. The time course of release of these cytokines parallels the evolution of the LPR, peaking at around 6 to 24 hours. The majority of Th2 cytokines appear to come from T cells. Corticosteroids (in the nose, lung and skin) inhibit the expression of mRNA for IL-4 and/or Il-5. In contrast, immunotherapy apparently upregulates Th1-type cytokines, particularly interferon-gamma. More precise therapy directed at these events (e. g. T cell-specific I. T., anti-IL-5 monoclonal antibodies, IL-4 and/or IL-5 receptor antagonists or soluble receptors, or even topical interferon-gamma or IL-12) may possibly be as effective as corticosteroids, with hopefully fewer side effects.

INTRODUCTION

This review will briefly describe the evolution of concepts concernig the pathophysiology of the allergic late phase reaction (LPR) in human skin and the possible role of T cells in this process. The study of the cutaneous LPR is of great interest since the skin provides a good model for studying basic molecular changes in allergic inflammation and, to a

certain extent, may reflect the allergic situation that occurs in less accessible target organs such as the nose or bronchi.

T LYMPHOCYTES IN NORMAL HUMAN SKIN

T cells are an important component of human skin. Although a few intraepidermal T cells can be detected by immunocytochemistry (ICC),[1] most T lymphocytes are located in the dermis,[2] where they have a preferential perivascular location, forming the dermal perivascular unit - D.P.U.[3] (Fig. 1) Most of these T lympchocytes are CD4+[3], express α and β chains of the T cell receptor (TCR), and have a "memory"-related phenotype.[4] It is known that migration of peripheral blood T cells into target organs is directed by the interaction between adhesion molecules on the lymphocyte surface (homing receptors) and their ligands on vascular endothelium (adressins). In normal human skin, 43% of T cells express the cutaneous lymphocyte-associated antigen (CLA), as defined by the monoclonal antibody HECA-452, whereas in non-cutaneous tissues the percentage of HECA-452+ T cells averages 5%,[5] and 16% in peripheral blood T cells.[6] CLA interacts with E-selectin (CD62E), expressed on cytokine--activated endothelial cells,[6] thus being one of the mechanisms that allows T cells migration into the skin.

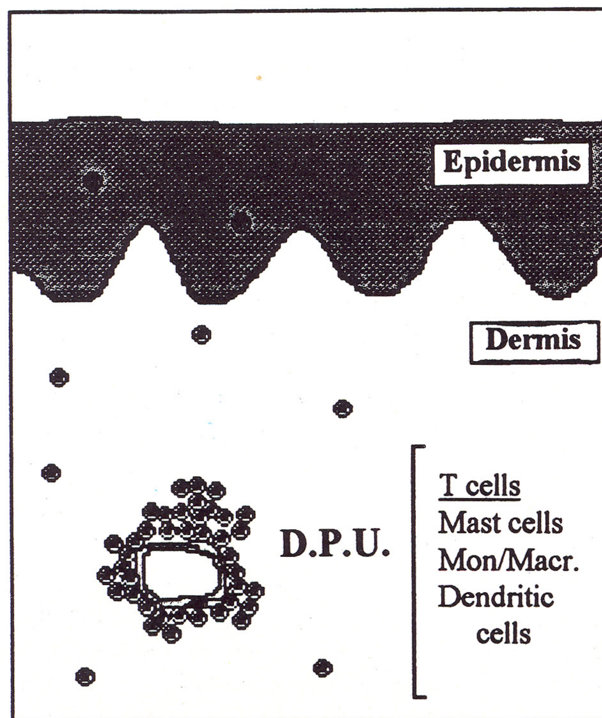

Figure 1

THE HUMAN CUTANEOUS ALLERGIC LATE-PHASE REACTION

Early studies

Charles Blackley (1873) was the first to describe the phenomenon of the allergic late phase reaction in the skin.[7] Being a hay fever sufferer, he induced a reaction in his forearm by abrading the skin and placing a grass pollen solution onto it. He developed an itching weal within minutes, which later developed uinto a swollen and indurated area that attained its maximum in 6 hours and which subsided in 48 hours - the late phase reaction (LPR).

Cooke, in 1922, injected himself with a horse dander-containing solution to which he was allergic. He then described the macroscopic features of the early phase reaction (EPR), consisting of a weal and flare response, and the later, more indurated lesion that was still present 24 hours after allergen challenge (the LPR).[8] Kline and collaborators took the next step in 1932, by analysing the histological reaction underlying the LPR.[9] These authors described the cellular infiltrate (eosinophils, neutrophils, basophils) and mentioned the presence of a mononuclear cell infiltrate. At this time, the only way to discriminate between different cell types was through routine staining, which did not allow the differentiation between monocytes and lymphocytes in tissues.

Although the LPR gained some attention after 1922, particularly in terms of histopathological description, the underlying pathophysiological mechanisms remained unknown. A growing interest in the possible classification of the LPR in one of the types of hypersensitivity described by Gell and Coombs[10] motivated some investigators to dissect the inflammatory reaction further. Noticing that most patients with allergic bronchopulmonary aspergillosis (ABPA) or with extrinsic allergic alveolitis (EAA) developed a dual "allergic" reaction (EPR and LPR) to *Aspergillus* antigens, Pepys et al. began in 1969 a systematic study of the cutaneous LPR. They collected biopsies for immunocytochemistry (ICC) after intradermal challenge.[11] These authors found high levels of IgG- and C3a/C5a-bearing immune complexes at the sites of inflammatory reactions. This suggested that the LPR might be a type III hypersensitivity, similar to the Arthus reaction. The fact, however, that the patients used for this study suffered from ABPA or EAA, made the interpretation of the LPR results very difficult since these diseases are associated with a type III pathology. The idea that the LPR might be caused by such a mechanism was, however, once

again put forward by Taylor and Shivalkar (1971), who found a relatively abundant complement deposition in arterioles, in the cutaneous LPR of allergic patients.[12]

IgE and the LPR

It was not until the discovery of IgE[13] and its role in the early phase reaction of cutaneous hypersensitivity to allergens[14], that new steps in clarification of the mechanisms underlying the LPR were taken. In 1973, Dolovich et al. supplied the first indication that IgE might indeed be an important factor for the development of the LPR.[15] Following injection of anti-IgE polyclonal antibodies (or their F (ab')2 fragments) intradermally, they were able to detect LPR that were macroscopically and microscopically similar to those induced by allergen. These authors also described the absence of complement or Ig deposition and were the first to identify the presence of lymphocytes at the inflammatory sites in addition to infiltration by eosinophils.

In 1976, Solley et al. confirmed the importance of IgE for the induction of the LPR, thereby implicating mast cells and basophils in the pathogenesis of this process. They were able to transfer the late cutaneous response (as well as the early response) using serum from an allergic donor to a non-allergic recipient. Passive transfer was lost when the serum was treated in various ways (heat, passing over a chromatographic column containing anti-IgE, etc.) which removed IgE antibody from donor serum. These authors also stressed that lymphocytes were an important cellular component of the inflammatory infiltrate.[16] Again, no significant complement or Ig deposition was found.

The idea that the LPR could be a form of type III hypersensitivity was gradually being cast away, since most authors could not find Ig or complement deposition. Frew and Kay, in 1991, studying post-challenge skin biopsies from twenty.four atopic patients with sensitivity to common aeroallergens again could not detect significant complement or Ig deposition.[17]

Cellular infiltration in the LPR

Following development of a wide range of polyclonal and monoclonal antibodies, attention was focused on cells that infiltrated the LPR. In an attempt to investigate the relationship between the different cells described in the LPR (namely eosinophils, neutrophils and mononuclear cells), Frew and Kay investigated the cutaneous LPR in atopic patients. Skin biopsies were collected at 6, 24 and 48 hours after allergen challenge. Increased numbers of T lymphocytes, most of which were CD4+, were detected by immunocytochemistry at the site of the LPR, at all time points. A small percentage of the infiltrating cells expressed interleukin-2 receptor (IL-2R+ or CD25+ cells), an indication of cell activation. Activated eosinophils were also detected in most biopsy specimens. At 24 hours after allergen challenge, there was a strong correlation between the number of CD4+ cells and activated eosinophils, which suggested an interaction between T cells and eosinophils, in the evolution of the cutaneous LPR.[18] In view of the fact that the IL-2 receptor (CD25) can be expressed by activated T-and non-T cells, Hamid et al performed double staining ICC for a pan-T cell marker (CD3) and CD25.[19] The results obtained showed that most CD25+ cells were also CD3+, suggesting that most activated LPR-infiltrating cells were indeed T lymphocytes. Although only about 10% of CD3+ cells were also CD25+, the potent biological actions of these cells suggest that even small numbers of activated T cells can intervene in the modulation of inflammatory processes.[20]

T cells expressing the γ and δ chains of the TCR are present in the human dermis and epidermis,[1] but their relevance to the cutaneous LPR is unknown at present.

Memory T lymphocytes in the cutaneous LPR

Leukocytes express a membrane glycoprotein (CD45) which may modelate the transduction of activating signals.[21,22] Expression of different isoforms of the restricted epitopes of CD45 allows the subdivision of T lymphocytes into two subtypes: CD45RA+ and CD45R0+. CD45R0+ T cells are considered to be "memory" T cells, since they express higher levels of adhesion molecules,[23] respond better to recall antigens,[24] and have a preferential recirculation pattern to target organs rather than to lymph nodes.[25] It therefore became important to characterise the subtype of T cells in the skin and whether the subtypes changed during allergen challenge. Bos et al showed, in 1989, that most CD4+ T cells in normal skin were CD45R0+,[4] and Frew et al. demonstrated, in 1991, that CD45R0+/CD4+ cells were significantly increased 6 and 24h after intradermal allergen challenge in atopic patients.[26]

T cells and cytokines in the cutaneous LPR

Activated T cells, particularly CD45R0+ cells, are capable of secreting a wide range of cytokines, which allow them to perform different functions. In 1987, Mosmann et al showed that murine T cells could be divided into two types according to their cytokine secretory pattern, *in vitro*. T helper 1 (Th1) cells produced IL-2 and IFNγ but not IL-4. In contrast, Th2 cells could produce IL-4 but not IFNγ or IL-2.[27] It was later shown that "*in vitro*" Th1 and Th2 cells have a wider range of cytokine-secreting capacities. Th2 cells can, for instance, also secrete IL-5.[28] Wieringa et al[29] and Romagnani et al[30] then extended these observations to humans, showing that atopic patients apparently have clonotypic imbalances between allergen-specific Th1 and Th2 cells, with a predominance of the latter subtype *in vitro*.

In humans, both Th1 and Th2 cells can secrete IL-3 and GM-CSF,[28] IL-13[31] and IL-10,[32] although the levels detected in the supernatants of *in vitro* cultures of each type of cell have been variable.

In order to investigate whether T cell-derived cytokines might be involved in the LPR, Kay et al used "*in situ*" hybridization to study cytokine mRNA in skin biopsies collected 24h after allergen challenge. These authors were the first to show that in the cutaneous LPR most of the lymphocytes present express mRNA for IL-3, IL-4 and GM-CSF, and not for IL-2 or IFNγ.[33] Indeed significant correlations were seen between IL-5 mRNA+ cells and IL-3, IL-4 and GM-CSF mRNA+ cells, at 24 hours. This indicates that most T cells infiltrating the LPR have a Th2-type of cytokine pattern and suggests that these T cells may modelate the involvement of other cells in the allergic process through the cytokines secreted (Table 1). IL.3 and GM-CSF, for instance, can act as histamine releasing factors for basophils.[34] IL-5, on the other hand, is known to be moderately chemotactic for eosinophils,[35] to promote the differentiation of mature eosinophils from precursor cells,[36,37] to prolong eosinophil survival,[38] to enhance eosinophil adhesion to endothelial cells[39] and to prime eosinophils for increased functional activity.[40] In adition, in a murine *in vivo* model, intradermal injection of Th2 clones into naive syngeneic mice induced an inflammatory swelling that peaked at 6 hours.[41] This reaction was abrogated by administration of anti-IL-4 monoclonal antibodies (mAb), thereby suggesting that IL-4 may also be of relevance for the inflammation in the LPR. In fact, IL-4 can act *in vitro* as a T cell growth factor[42] and is capable of inducing the expression of the adhesion molecule vascular cell adhesion molecule - 1 (VCAM-1; CD106) on endothelial cells.[43] This may allow eosinophils and T cells, which express the ligand for VCAM-1 (VLA-4 or very late activation antigen 4; CD49d) to migrate into sites of allergic inflammation.

Finally, IL-4 and the recently described IL-13, secreted by Th2 cells induce isotype switching in favour of IgE secretion *in vitro*.[44,45]

Although some of these cytokines may be produced by other cells such as mast cells or basophils (IL-4,[46] IL-5,[47]) or eosinophils (IL-3, GM-CSF,[48]

TABLE 1

Cytokine	Target cells	*In vitro* actions
IL-3/IL-5/ /GM-CSF	Eosinophils	- growth, maturation and differentiation
		- priming, activation and mediator release
		- enhanced viability
		- terminal differentiation (IL-5)
	Basophils	- activation and histamine release
		- IgE-dependent IL-4 production
		- increased integrin expression (CD18)
	B cells	- co-factor for IgE synthesis (IL-5)
IL-4	T cells	- differentiation towards Th2-type cells
		- T cell growth factor
	B cells	- B cell growth factor
		- Ig switch to IgE
		- increases HLA-II and CD23 expression
		- increases IL-6 production
	Monocytes	- increases HLA-II and CD23 expression
	Endothelial cells	- increases VCAM-1 expression
IL-6	B cells	- terminal differentiation
		- amplification of IgE production
	T cells	- co-factor for early steps of activation
IL-10	B cells	- growth factor
	Macrophages	- inhibits HLA-II expression (and APC function)
		- inhibits cytokine secretion
IL-13	B cells	- Ig switch to IgE
		- increases HLA-II and CD23 expression
		- growth factor
	Mon./Macrophages	- increases HLA-II and CD23 expression
RANTES	Eosinophils	- chemoattraction and activation
	Basophils	- histamine release
	T cells	- chemoattractant for "memory" T cells

IL-5[49]), the fact that atopic patients generally have high levels of IgE specific for one or relatively few allergens suggests that an imbalance at the level of allergen-specific cells (particularly T cells) may be critical for the development of allergic responses.

T cell migration into LPR sites

The process of T cell migration into the skin is enhanced in inflammatory situations. For example, the percentage of HECA-452+ T cells (CLA+) in cutaneous inflammation, the percentage hardly ever increases above 5%. Since CLA is preferentially expressed on "memory" T cells and has been shown to interact with E-selectin on activated endothelial cells,[6] this suggests that T cell: endothelial cell interactions are important for homing of T cells into specific target organs, by being decisive in the first step of transendothelial migration. Since E-selectin is also expressed in other organs, other specific interactions may also be involved. To our knowledge, expression of HECA-452 has not been studied in the LPR infiltrate.

Other interactions between T cells and the endothelium (through selectins and integrins) may be involved in the early steps of T cell transendothelial migration into normal and, particularly, inflamed skin. Integrin-mediated interactions are an important second step in migration into tissues. T cells express CD11/CD18 (particularly LFA-1), which binds the intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 (CD102) on endothelial cells.[50,51] Another integrin, the very late activation antigen-4 (VLA-4; CD49d) interacts with the vascular cell adhesion molecule-1 (VCAM-1; CD106), thereby enabling T cells to traverse the endothelium.[52] Inflammation in the skin upregulates the expression of adhesion molecules on the local endothelium,[53] thereby amplifying infiltration of cells into the tissue.

T cell interactions with other cells and the extracellular matrix in cutaneous LPR

T cells possess surface molecules that bind ligands expressed on extracellular matrix (ECM) proteins in the skin. These interactions are important for the intra-tissue migration and preferential localization of T cells in the dermis. As shown in Fig. 2, several integrins on T cells bind epitopes on collagen and fibronectin.[54] Interestingly, the perivascular ECM in the dermis appears to have the highest fibronectin content in adult human skin.[54]

In addition, T cells can also establish direct contact with cells that can act as antigen-presenting cells (APC), such as Langerhans cells and keratinocytes,[55,56] namely through interactions between LFA-1, the T cell receptor and CD2 (on T cells) and ICAM-1, HLA-DR and LFA-3, respectively (on keratinocytes).[57]

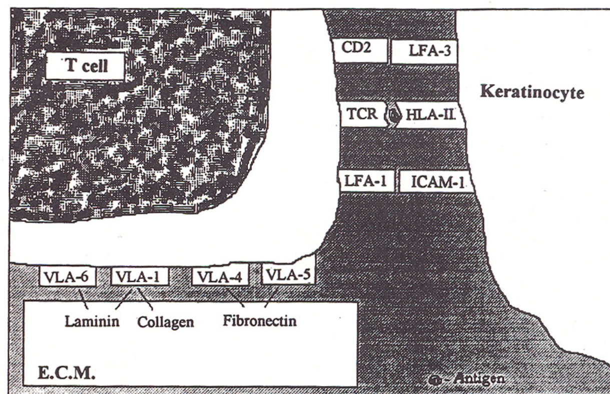

Fig. 2

Cell-mediated type of hypersensitivity and cutaneous LPR

The role of type IV hypersensitivity, as defined by Gell and Coombs,[10] in cutaneous LPR is unclear at present. In order to assess the importance (or otherwise) of the cellular infiltration in the LPR, Gaga et al compared the LPR with a typical cell--mediated hypersensitivity lesion (Type IV - classical delayed type hypersensitivity/DTH), as reflected by intradermal injection of protein purified derivate (PPD).[58] Biopsies were taken at 24 and 48 hours and were analysed by immunocytochemistry. In general cell numbers in the LPR were 2-4 times less than those evoked by PPD. T cells were prominent in both the LPR and DTH. Most of these T cells were CD4+ (helper) lymphocytes. Eosinophil activation was observed in LPR and, to a much lesser extent, also in the DTH. These findings suggested that the LPR was, to some extent, a form of cell-mediated hypersensitivity although the observed T cell kinetics were different from those seen with the classical DTH.

In order to better dissect the different cellular kinetics between DTH and LPR and to evaluate whether these were associated with different patterns of cytokine release, Tsicopoulos et al studied the kinetics of both processes with further detail and

assessed both cellular infiltration and cytokine release.[59] Again, it was found that both DTH and LPR have an important infiltration of CD3+ T cells, most of which are CD4+. In DTH the maximal numbers were observed at 48 hours, whereas in the LPR, the infiltrate paralleled the clinical response and was maximal at 6 hours. Eosinophils and neutrophils also infiltrated the LPR with the same type of kinetics, but did not significantly increase in DTH. In addition, whereas both the LPR and the DTH seemed to have a release peak of the Th1-type of cytokines at 48 hours, only the LPR showed an earlier (6h) peak of the Th2-type of cytokines, which may be involved in the pathophysiology of this process.

Allergen-specificity of T cells in the cutaneous LPR

Although activated T cells have been shown to be present in the cutaneous LPR and to express mRNA for cytokines that may be crucial for the pathogenesis of this response, the proportion of infiltrating T cells which are allergen-specific is unknown. Allergen--specific T cell cloning frequency and T cell clone culture studies have given an indication of the presence of allergen-specific T cells in the lesional skin of patients with atopic dermatitis,[60] with allergic contact dermatitis[61] and in peripheral blood and bronchoalveolar lavage fluid from asthmatic patients.[62,63] The frequency of allergen-specific T cells in the cutaneous LPR has not, however, been determined. We are about to address this problem by attempting to clone T cells from skin biopsies in an allergen-specific fashion.

Modulation of the LPR: influence on macroscopic and cellular characteristics

We are presently trying to modulate the intensity of the cutaneous LPR in an attempt to correlate differences found in the macroscopic size of the lesion with changes in the underlying histological features. To do that, we are performing time-course and dose-response experiments with repetitive cutaneous allergen challenge and intradermal injection of recombinant human cytokines (IL-4, IL-5 and IFNγ). We are studying biopsies from the LPR by single and double immunocytochemistry, to evaluate cell phenotype and intracellular cytokine product, "*in situ*" hybridization to study cytokine mRNA expression at a cellular level and reverse-transcription followed by polymerase chain reaction amplification (RT-PCR), to study overall levels of cytokine mRNA expression in skin biopsies. This will allow us to gain further insight into the pathophysiology of the allergen-induced LPR and will eventually allow the use of a more focused therapy. Indeed, many groups apart from us have reported several therapeutic approaches that have been shown to interfere with the activation and/or proliferation of T cells "*in vitro*". In addition, some also affec t the infiltration and activation of T lymphocytes in the cutaneous late phase reactions.

Corticosteroids

Corticosteroids (CS) are capable of interfering with T cell activation *in vitro*. Indeed, CS inhibit the transcription of the IL-2 and IFNγ,[64] IL-4[65] and IL-5 genes.[66] *In vivo*, CS have been shown to be potent inhibitors of the cutaneous LPR.[67] Prednisolone, for instance, given in a single dose of 20 mg was shown to significantly reduce the macroscopic magnitude of the cutaneous LPR and to inhibit the infiltration of CD45+ (total leukocytes) and CD25+ cells. In addition, it also inhibited, although not attaining statistical significance, the percentage of CD3+, CD4+ and CD8+ cells in the LPR.[68] This study showed that a single dose of CS may inhibit the activation of T cells present in the LPR, although it may not alter the total number of T lymphocytes. Our preliminary data from a double-blind, placebo-controlled cross-over study where we performed a five-day course of prednisolone (20 mg/day) showed a significant decrease on eosinophil infiltration and activation and a moderate, although non-significant, reduction in the numbers of infiltrating T cells. In addition, prednisolone treatment was also associated with a significant decrease in allergen-induced expression of IL-5 mRNA (a reduction in the expression of IL-4 mRNA was pratically significant, p=0.054, Mann-Whitney U test). These results confirm those of Robinson et al in the lung, where prednisolone was shown to inhibit expression of mRNA for IL-4 and IL-5,[69] and those of Masuyama et al who reported that topical administration of fluticasone propionate inhibits IL-4 mRNA expression in the nasal LPR.[70]

Cyclosporin A and FK506

Besides having inhibitory effects on eosinophil and neutrophil cytokine release,[48] cyclosporin A (CyA) and FK506 may abrogate T cell activation and proliferation *in vitro*, by inhibiting transcription of the IL-2 gene.[71,72] In addition, these drugs have been reported to inhibit the transcription of other

early activation genes such us IL-3, IL-4, GM-CSF and IFNγ, and to downregulate the expression of the IL-2 receptor α-chain, at posttranscriptional level.[73] The effect in the cutaneous LPR has not been studied.

Anti-histamines

The effects of antihistamines on the cutaneous LPR are controversial. Kontou-Fili et al reported that cetirizine significantly inhibited cutaneous reactions even 6 hours after cutaneous challenge with histamine.[74] Effects of anti-histamines on the allergen-induced LPR have, however, shown a high degree of variability. Although a few studies showed that some of these drugs (e.g. chlorpheniramine, astemizol) do not inhibit the size of the allergen--induced LPR,[74,75] our own experience and that of others, using modern potent H1-selective drugs is that for example clemastine and cetirizine may have a slight to moderate effect.[68,76,77] Curiously, H-2 antihistamines (cimetidine) given together with H-1 antihistamines (chlorpheniramine) were shown to significantly inhibit the macroscopic size of the LPR.[75] There is evidence to suggest that H-2 antihistamines can counteract *in vitro* histamine--induced downregulation of the Th1-type of cytokines (IL-2 and IFNγ)[79] and upregulation of ICAM-1 on epidermal keratinocytes.[80] These effects of H-2 antihistamines may be important for an eventual action *in vivo*.

Immunocytochemical studies performed on skin biopsies from the LPR have not shown a significant inhibitory effect of cetirizine (20 mg, single dose) on the allergen-induced T cell infiltration or activation in the LPR.[68]

Immunotherapy (I.T.)

It has long been known that I.T. can suppress the cutaneous allergen-induced LPR.[81,82] Most of the results in the effects of I.T. on T lymphocytes have, however, most often been obtained from studies of circulating T cells.

In 1979, Canonica et al analysed peripheral blood T lymphocytes by flow cytometry and described a relative deficiency of CD8+ T cells in atopic patients. Further, these authors showed that clinically successful I.T. induced an increase in the relative percentage of CD8+ T cells.[83]

Rocklin et al, in 1980, extended these observations by demonstrating that I.T. suppressed allergen--induced proliferation of peripheral blood T cells and that this was associated with an increase in CD8+ T cells which functionally suppressed allergen-specific T lymphocyte proliferation *in vitro*.[84]

In 1984, Hsieh showed that allergen-induced proliferation of peripheral blood CD4+ T cells was lower and that of CD8+ T cells was higher after I.T., as compared to pre-I.T. values.[85] In additon, it was also shown that T cells produced and responded less to IL-2 after I.T..[86] These reports suggest that I.T. may induce the appearance of CD8+ T cells that have the capacity to downregulate the response to specific allergens, either trough cytolysis of antigen--presenting cells or through the release of cytokines such as IFNγ.[87]

Varney et al studied a group of 40 adult seasonal rhinitic patients in order to evaluate the influence of grass pollen I.T. on the cellular infiltration and cytokine mRNA expression during allergen-induced cutaneous LPR.[88] Clinical improvement was accompanied by a decrease (64%) in the size of the LPR. Biopsies were taken 24 hours after allergen--challenge and used for ICC and *in situ* hybridization for IL-2, IFNγ, IL-3, IL-4, IL-5 and GM-CSF mRNA. Clinically successful I.T. was associated with a reduction in the total number of leucocytes (CD45+) and T lymphocytes (CD3+). There was a decrease in CD4+ T cells and a trend for a decrease in the number of eosinophils. Surprisingly, a higher percentage of activated cells (CD25+ or HLA-DR+) was also observed. Interestingly, there were significant increases in IL-2 and IFNγ mRNA and probably a decrease (non significant) in the median cell counts for IL-3, IL-4, IL-5 and GM-CSF after I.T.. Finally, there was a positive correlation between IL-2 mRNA+ and CD25+ cells. Since most CD25+ T cells in the LPR have been shown to be T cells (CD3+),[19] these results suggest that I.T. may upregulate the expression of a Th1 pattern of cytokines that may be involved in the induction of allergen-specific T cell tolerance.

More specifically targeted I.T. will provide further insight into mechanisms underlying the LPR. This may involve the administration of allergen-derived peptide sequences that are only recognized by T cells, in the context of MHC class II presentation on APC, and not by IgE.[89] In one study, T cell epitopes from the cat allergen *Fel d I* were administered to mice. Allergen-specific T cell tolerance could be obtained in this way.[90] These epitopes - IPC-1 and IPC-2, have also been given to patients allergic to cat dander, and preliminary results show that this form of I.T. may have a lower occurence of

severe anaphylactic reactions. Curiously, some of the patients developed a cutaneous LPR 6 to 8 hours after intradermal injection of the peptides.[91] This is an additional reason to suggest that T lymphocytes are indeed crucial for the pathogenesis of the cutaneous LPR.

REFERENCES

1. **Bos JD, Teunissen MB, Cairo I et al.** T cell receptor gamma delta bearing cells in normal human skin. *J Invest Dermatol* 1990; 94: 37-42.
2. **Bos JD, Zonneveld I, Das PK, Krieg SR, Van der Loos CM, Kapsenberg ML.** The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. *J Invest Dermatol* 1987; 88:569-73.
3. **Bos JD, Das PK, Kapsenberg ML.** Lymphocyte subsets of the skin immune system (SIS). In Caputo R, ed *Immunodermatology*, Rome: CIC Edizioni Internazionali 1987; 67-70.
4. **Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg ML.** Predominance of "memory" T cells (CD4+, CDw29+) over "naive" T cells (CD4+, CD45R+) in both normal and diseased human skin. *Arch Dermatol Res* 1989; 281: 24-30.
5. **Bos JD, De Boer OJ, Tibosch E, Das PK, Pals ST.** Skin--homing T lymphocytes: detection of cutaneous lymphocyte--associated antigen (CLA) by HECA-452 in normal human skin. *Arch Dermatol Res* 1992; 285: 179-83.
6. **Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC.** ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 1991; 349: 796-9.
7. **Blackley CH.** Experimental researches on the causes and nature of catarrhus aestivus (hay fever or hay asthma). *London; Bailliere Tindall and Cox* 1873: 84-6.
8. **Cooke RA.** Studies in specific hypersensitiveness. IX. On the phenomenon of hypersensitization (the clinically lessened sensitiveness of allergy). *J Immunol* 1922; 7: 219-24.
9. **Kline BS, Cohen MB, Rudolph JA.** Histologic changes in allergic and nonallergic wheals. *J Allergy* 1932; 3: 351-55.
10. **Coombs RRA, Gell PGH.** Classification of allergic reactions responsible for clinical hypersensitivity and disease. *In: Coombs RRA, Gell PGH, eds Clinical Aspects of Immunology. Oxford: Blackwell Scientific Publications* 1968: 317-37.
11. **Pepys J.** Immunopathology of allergic lung disease. *Clin Allergy* 1973; 3: 1-22.
12. **Taylor G, Shivalkar PR.** "Arthus-type" reactivity in the nasal airways and skin in pollen sensitive subjects. *Clin Allergy* 1971; 1: 407-14.
13. **Bennich H, Ishizaka K, Johansson SGO, Rowe DS, Stanworth DR, Terry WD.** Immunoglobulin E, a new class of human immunoglobulin. *Immunocytochemistry* 1968; 5: 327-8.
14. **Ishizaka K, Ishizaka T.** Induction of erythema-wheal reactions by soluble antigen gammaE-antibody complexes in humans. *J Immunol* 1968; 101: 68-78.
15. **Dollovich J, Hargreave FE, Chalmers R, Shier KJ, Gauldie J, Bienenstock J.** Late cutaneous allergic responses in isolated IgE-dependent reactions. *J Allergy Clin Immunol* 1973; 52: 38-46.
16. **Solley GO, Gleich GJ, Jordon RE, Schroeter AL.** The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. *J Clin Invest* 1976; 58: 408-20.
17. **Frew AJ, Kay AB.** Failure to detect deposition of complement and immunoglobulin in allergen-induced late-phase skin reaction in atopic subjects. *Clin Exp Immunol* 1991; 85: 70-4.
18. **Frew AJ, Kay AB.** The relationship between infiltrating CD4+ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J Immunol* 1988; 141: 4158-64.
19. **Hamid Q, Barkan J, Robinson DS, Durham SR, Kay AB.** Co-expression of CD25 and CD3 in atopic allergy and asthma. *Immunology* 1992; 75: 659-63.
20. **Tomkinson BE, Wagner DK, Nelson DL, Sullivan JL.** Activated lymphocytes during acute Epstein-Barr virus infection. *J Immunol* 1987; 139: 3802-7.
21. **Thomas ML, Lefrancois L.** Differential expression of the leukocyte-common antigen family. *Immunol Today* 1988; 9: 320-6.
22. **Ostergaard HL, Trowbridge IS.** Coclustering CD45 with CD8 alters the phosphorylation and kinase activity of $p56^{lck}$. *J Exp Med* 1990; 172: 347-50.
23. **Akbar AN, Salmos M, Janossy G.** The synergy between naive and memory T cells during activation. *Immunol Today* 1991; 12: 184-8.
24. **Sanders ME, Makgoba MW, Shaw S.** Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subjects. *Immunol Today* 1988; 9: 195-9.
25. **Mackay CR.** Homing of naive, memory and effector lymphocytes. *Curr Opin Immunol* 1993; 5: 423-7.
26. **Frew AJ, Kay AB.** UCHL1+ (CD45R0+) "memory" T cells predominate in the CD4+ cellular infiltrate associated with allergen-induced late-phase skin reactions in atopic subjects. *Clin Exp Immunol* 1991; 84: 270-4.
27. **Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL.** Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136: 2348-57.
28. **Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR.** Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays and monoclonal antibodies. *J Exp Med* 1987; 166: 1229-44.
29. **Wierenga EA, Snoek M, De Groot C et al.** Evidence for compartmentalisation of functional subsets of CD4+ T-lymphocytes in atopic patients. *J Immunol* 1990; 144: 4651-6.
30. **Parronchi P, Macchia D, Piccini M-P et al.** Allergen and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci USA* 1991; 88: 4538-42.
31. **Zurawski G, De Vries JE.** Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today* 1994; 15: 19-26.
32. **Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S.** Human IL-10 is produced by both Type 1 helper (Th1) and Type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993; 150: 353-60.
33. **Kay AB, Ying S, Varney V et al.** Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991; 173: 775-8.
34. **Haak-Frendscho M, Arai N, Arai K, Baeza ML, Finn A, Kaplan AP.** Human recombinant granulocyte-macrophage colony-stimulating factor and interleukin 3 cause basophil histamine release. *J Clin Invest* 1988; 82: 17-20.

35. **Wang AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB.** Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988; 137: 62-9.

36. **Sanderson CJ, Warren DJ, Strath M.** Identification of a lymphokine that stimulates eosinophil differentiation in vitro. Its relationship to interleukin-3 and functional properties of eosinophils produced in cultures. *J Exp Med* 1985; 162: 60-74.

37. **Campbell HD, Tucker WQJ, Short Y et al.** Molecular cloning, nucleotide sequence and expression of the gene encoding human eosinophil differentiation factor (interleukin-5). *Proc Natl Acad Sci USA* 1987; 84: 6629-33.

38. **Rothenberg ME, Petersen J, Stevens RL et al.** IL-5 dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity and sustained antibody-dependent cytotoxicity. *J Immunol* 1989; 143: 2311-6.

39. **Walsh GM, Hartnell A, Mermod J-J, Kay AB, Wardlaw AJ.** Human eosinophil, but not neutrophil adherence to IL-1 stimulated HUVEC is a4b1 (VLA-4) dependent. *J Immunol* 1991; 146: 3419-23.

40. **Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA.** Recombinant human interleukin-5 is a selective activator of eosinophil function. *J Exp Med* 1988; 167: 219-24.

41. **Muller KM, Jaunin F, Masouye I, Saurat J-H, Hauser C.** Th2 cells mediate IL-4-dependent local tissue inflammation. *J Immunol* 1993; 150: 5576-84.

42. **Spits H, Yssel H, Takebe Y et al.** Recombinant interleukin-4 promotes the growth of human T cells. *J Immunol* 1987; 139: 1142-7.

43. **Schleimer RP, Sterbinsky SA, Kaiser J et al.** IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 1992; 148: 1086-92.

44. **Pene J, Rousset F, Briere F et al.** IgE production by human B cells is induced by IL-4 and suppressed by interferons γ, α and prostaglandin E2. *Proc Natl Acad Sci USA* 1988; 85: 8166-70.

45. **Punnonen J, Aversa G, Cocks BG et al.** Interleukin 13 induces interleukin-4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 1993; 90: 3730-4.

46. **Seder RA, Paul WE, Ben-Sasson SZ et al.** Production of interleukin-4 and other cytokines following stimulation of mast cell lines and in vivo mast cells/basophils. *Int Arch Allergy Appl Immunol* 1991; 94: 137-40.

47. **Bradding P, Feather IH, Wilson S et al.** Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5 and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; 151: 3853-65.

48. **Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS, Gleich GJ.** Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human periheral blood eosinophils and neutrophils. *J Exp Med* 1991; 174: 745-8.

49. **Desreaumaux P, Janin A, Dubucquoi S et al.** Synthesis of interleukin-5 by activated eosinophils in patients with eosinophilic heart diseases. *Blood* 1993; 82: 1553-60.

50. **Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA.** Induction by IL-1 and interferon-γ: tissue distribution, biochemistry, function of a natural adhesion molecule (ICAM-1). *J. Immunol* 1986; 137: 245-54.

51. **Nortamo P, Li R, Renkonen R et al.** The expression of human intercellular adhesion molecule-2 is refractory to inflammatory cytokines. *Eur J Immunol* 1991; 21: 2629-32.

52. **Graber N, Gopal TV, Wilson D, Beall LD, Polte T, Newman W.** T cell bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. *J Immunol* 1990; 145: 819-30.

53. **Parish WE.** Inflammation. In Champion ER, Burton JL, Ebling FJG, eds Rook, Wilkinson, Ebling Textbook of Dermatology. Oxford. Blackwell Scientific Publications 1992: 234-7.

54. **Couchman JR, Austria MR, Woods A.** Fibronectin-cell interactions. *J Invest Dermatol* 1990; 94: 7S-14S.

55. **Bjercke S, Elgo J, Braathen L, Thorsby E.** Enriched epidermal Langerhans cells are potent antigen-presenting cells for T cells. *J Invest Dermatol* 1984; 83: 286-9.

56. **Nickoloff BJ, Mitra RS, Green J, Shimizu Y, Thompson C, Turka LA.** Activated keratinocytes present bacterial-derived superantigens to T lymphocytes: relevance to psoriasis. *J Dermatol Sci* 1993; 6: 127-33.

57. **Nickoloff BJ, Mitra RS, Green J et al.** Accessory cell function of keratinocytes for superantigens: dependence on lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. *J Immunol* 1993; 150: 2148-59.

58. **Gaga M, Frew AJ, Varney V, Kay AB.** Eosinophil activation and T lymphocyte infiltration in allergen-induced late-phase skin reactions and classical delayed-type hypersensitivity. *J Immunol* 1991; 147: 816-22.

59. **Tsicopoulos A, Hamid Q, Jacobson MR et al.** Kinetics of cell infiltration and cytokine messenger RNA expression after intradermal challenge with allergen and tuberculin in the same atopic individuals. Submetido a *J Allergy Clin Immunol.*

60. **Van der Heijden F, Wierenga EA, Bos JD, Kapsenberg ML.** High frequency of IL-4-producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991; 97: 389-94.

61. **Kapsenberg ML, Res P, Bos JD, Schootemijer A, Teunissen MBM, Van Schooten W.** Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirements of dendritic antigen--presenting cell subsets. *Eur J Immunol* 1987; 17: 861-5.

62. **O'Hehir RE, Young DB, Kay AB, Lamb JR.** Cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite): a comparison of T- and B- cell antigen recognition. *Immunology* 1987; 62: 635-40.

63. **Hol BEA, Krouwels FH, Bruinier B et al.** Cloning of T lymphocytes from bronchoalveolar lavage fluid. *Am J Respir Cell Mol Biol* 1992; 7: 523-30.

64. **Arya SK, Wong-Staal F, Gallo RC.** Dexamethasone-mediated inhibition of human T cell growth factor and gamma-interferon mRNA. *J Immunol* 1984; 133: 273-6.

65. **Wu CY, Fargeas C, Nakajima T, Delespesse G.** Glucocorticoids suppress the production of interleukin-4 by human lymphocytes. *Eur J Immunol* 1991; 21: 2645-7.

66. **Rolfe FG, Hughes JM, Armour CL, Sewell WA.** Inhibition of interleukin-5 gene expression by dexamethasone. *Immunology* 1992; 77: 494-9.

67. **Poothullil J, Umemoto L, Dolovich J, Hargreave FE, Day RP.** Inhibition by prednisone of late cutaneous allergic responses induced by anti-serum to human IgE. *J Allergy Clin Immunol* 1976; 57:164-7.

68. **Varney V, Gaga M, Frew AJ, De Vos C, Kay AB.** The effect of a single oral dose of prednisolone or cetirizine on inflammatory cells infiltrating allergen-induced cutaneous late-phase reactions in atopic subjects. *Clin Exp Allergy* 1992; 22: 43-9.

69. **Robinson D, Hamid Q, Ying S et al.** Prednisolone treatment in asthma is associated with modulation of broncho-alveolar lavage cell interleukin-4, interleukin-5, and interferon-γ cytokine gene expression. *Am Rev Respir Dis* 1993; 148: 401-6.

70. **Masuyama K, Jacobson MR, Rak S et al.** Topical glucocorticosteroid (fluticasone propionate) inhibits cytokine mRNA expression for interleukin-4 (IL-4) in the nasal mucosa in allergic rhinitis. *Immunology* 1994 (em publicação).

71. **Henderson DJ, Naya I, Bundick RV, Smith GM, Schmidt JA.** Comparison of the effects of FK506, cyclosporin A and rapamycin on IL-2 production. *Immunology* 1991; 73: 316-21.

72. **Tocci MJ, Sigal NH.** Recent advances in the mechanism of action of cyclosporin and FK506. *Curr Opin Nephrol Hypert* 1992; 1: 236-40.

73. **Tocci MJ, Matkovich DA, Collier KA et al.** The immunosuppressant FK506 selectively inhibits expression of early T cell activation genes. *J Immunol* 1989; 143: 718-26.

74. **Kontou-Fili K, Paleologos G, Herakleous M.** Suppression of histamine-induced skin reactions by loratadine and cetirizine diHC1. *Eur J Clin Pharmacol* 1989; 36: 617-9.

75. **Smith JA, Mansfield LE, DeShazo RD et al.** An evaluation of the pharmacologic inhibition of the immediate and late cutaneous reaction to allergen. *J Allergy Clin Immunol* 1980; 65: 118-21.

76. **Bierman CW, Maxwell D, Rytina E et al.** Effect of H1-receptor blockade on late cutaneous reactions to antigen: a double-blind, controlled study. *J Allergy Clin Immunol* 1991; 87: 1013-9.

77. **Aas K.** Effects on ketotifen and clemastine on passive transfer of reaginic reaction. *Allergy* 1979; 34: 121-4.

78. **Michel L, De Vos C, Rihoux J-P et al.** Inhibitory effect of oral cetirizine on in vivo antigen-induced histamine and PAF--acether release and eosinophil recruitment in human skin. *J Allergy Clin Immunol* 1988; 82: 101-9.

79. **Dohlsten M, Sjogren HO, Carlsson R.** Histamine acts directly on human T cells to inhibit interleukin-2 and interferon--gamma production. *Cell Immunol* 1987; 109: 65-74.

80. **Mitra RS, Shimizu Y, Nickoloff BJ.** Histamine and *cis*--urocanic acid augment tumor necrosis factor-alpha mediated induction of keratinocyte intercellular adhesion molecule-1 expression. *J Cell Physiol* 1993; 156: 348-57.

81. **Pienkowksi MM, Norman PS, Lichtenstein LM.** Suppression of late-phase skin reactions by immunotherapy with ragweed extract. *J Allergy Clin Immunol* 1985; 76: 729-34.

82. **Fling JA, Ruff ME, Parker WA et al.** Suppression of the late cutaneous response by immunotherapy. *J Allergy Clin Immunol* 1989; 83: 101-9.

83. **Canonica GW, Mingau MC, Milioli G et al.** Imbalances of T cell subpopulations in patients with atopic diseases and effect of specific immunotherapy. *J Immunology* 1979; 123: 2669-72.

84. **Rocklin RE, Sheffer AL, Greineder DK, Melmon KL.** Generation of antigen-specific suppressor cells during allergy desensitization. *New Engl J Med* 1980; 302: 1213-9.

85. **Hsieh K-H.** Changes of lymphoproliferative responses of T cells subsets to allergen and mitogen after hyposensitization in asthmatic children. *J Allergy Clin Immunol* 1984; 74: 34-40.

86. **Hsieh K-H.** Altered interleukin-2 (IL-2) production and responsiveness after hyposensitization to house dust. J *Allergy Clin Immunol* 1985; 76: 188-94.

87. **Salgame P, Abrams JS, Clayberger C et al.** Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science* 1991; 254: 279-82.

88. **Varney VA, Hamid QA, Gaga M et al.** Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993; 92: 644-51.

89. **O'Hehir RE, Lamb JR.** The cellular and molecular basis of the human T cell response to *Dermatophagoides spp* (house dust mite). *Parasite Immunol* 1991; 13: 109-19.

90. **Briner TJ, Kuo M-C, Keating KM, Rogers BL.** Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. *Proc Natl Acad Sci USA* 1993; 90: 7608-12.

91. **Norman PS.** Modern concepts of immunotherapy. *Curr Opin Immunol* 1993; 5: 968-73.