

KINETICS AND DYNAMIC EVALUATION OF SPECIFIC IMMUNOTHERAPY

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SUMMARY: Specific immunotherapy (SIT) is frequently used in the treatment of allergic diseases. However, the mechanisms by which SIT achieves clinical improvement remained unclear. We decided to study the in vivo kinetics of this therapy, using a nuclear medicine approach (leukocytes labelled with ^{99m}Tc -HMPAO) in patients on maintenance doses of specific immunotherapy with confirmed clinical efficacy.

Material and methods: We studied 13 allergic patients grouped according to different treatment schedules: subcutaneous aqueous allergenic extract (3 latex and 2 hymenoptera venom), subcutaneous depot extract (2 house dust mite and 2 pollens), subcutaneous modified allergens (2 pollens), sublingual extract (2 house dust mites). The control group included two allergic patients submitted to subcutaneous injections of bacterial extract (1 patient - positive control), and aqueous solution (1 patient). At the same time that the therapeutic allergen was administered subcutaneously, the autologous labelled white cells were injected intravenously in a peripheral vein in the contralateral arm. A thoracic dynamic acquisition of 60 mins, 64x64 matrix, 2 frame/min, in anterior view was performed. Static acquisition for 256x256 matrix, during 5 mins each at 60, 90, 120, 180, 240, 300 and 360 mins after the administration of the radiolabelled leukocytes, in thoracic (anterior and posterior), and abdominal view were performed. During the examination, the local erythema was monitored. A similar procedure was undertaken for sublingual administration of immunotherapy.

Results: The inflammatory activity at the site of SIT injection (aqueous depot extract) started in the first hour and the increase was time related. For modified allergen extract and sublingual SIT the activity was present since the beginning of the administration. The ascendant lymphatic drainage, which was directed to the homolateral axillary region, to the lymphoid tissue of the upper mediastinum and to the anterior region of the neck began earlier. Thoracic focalisations were present for all the patients, whereas bowel focalisations were only observed for the subcutaneous route of administration. Sublingual SIT did not induce axillary or intestinal inflammatory focalisations, even though the patients had swallowed the allergenic extract. The uptake coefficient in individualized areas corrected to the uptake coefficient background was also studied.

Conclusions: For the subcutaneous route of administration, except for gluteraldehyde modified allergen, the local inflammatory activity at the allergenic injection site was significantly higher in depth and was time dependent, maintaining activity even after complete disappearance of the erythema and/or wheal. These results express a prompt inflammatory involvement of the immune system with this allergenic therapy, which was unexpected until now. We also observed differences concerning allergic diseases, the type of allergenic extracts and routes of administration.

Key-words: Specific immunotherapy - Subcutaneous immunotherapy - Sublingual immunotherapy - Allergic inflammation - Radio labelled cells - Scintigraphy, ^{99m}Tc -HMPAO.

INTRODUCTION

Allergen specific immunotherapy (SIT) and total avoidance of exposure to allergens, to which the patient is sensitised, are the only interventions that really influence in the long-term control of the allergic disease (1, 2).

In fact, it is now obvious that SIT is the only curative treatment for hymenoptera venom allergy or latex anaphylaxis, two examples of life-threatening IgE dependent mechanisms (1-4). A lot of DBPC studies (double-blind, placebo-controlled) provide longitudinal data on the clinical efficacy of subcutaneous SIT on mite, pollen, epithelium and some mould species. For other respiratory allergic diseases such as bronchial asthma, allergic rhinitis and conjunctivitis, the reduction of symptom scores/need for medication were also observed (5).

Allergen vaccines are, in fact, the only treatment that may affect the natural course of allergic disease, preventing the development of asthma in patients with allergic rhinitis, reducing the severity of the disease

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and the need for anti-allergic drugs thereby improving the patient's quality of life (1). It is also now recognized that immunotherapy is long-lasting effective and should be started earlier in the course of the disease. It has been proposed that SIT reduce the risk of development of new sensitisations (6, 7), but recent data has not confirmed this protective effect on monosensitised allergic patients (8).

There are several factors that are important to the clinical outcome of SIT concerning efficacy and tolerance: the use of premedication, the allergen source, the purification, the standardization and the stability of the extract, the pharmaceutical presentation of the allergen (native or chemically modified; aqueous or depot). The clinical manifestations before and during the treatment, the route of SIT administration, and the schedule followed, should also be evaluated (9).

The aqueous extracts are highly effective, but induce more side-effects (local and systemic) than depot and modified vaccines. These have been developed in an attempt to reduce or remove allergenicity, while preserving or increasing the immunogenicity. Aqueous subcutaneous SIT is now the gold standard treatment for hymenoptera venom allergy and depot aeroallergen extracts for respiratory allergy. The use of chemically modified allergens is not consensual. Although recent papers demonstrate a clinical benefit with allergoid therapy, DBPC studies comparing both extracts are still missing (10).

Local immunotherapy is another strategy for allergic disease treatment. In spite of the therapeutic benefit observed in some studies, there is no consensus on the use of the nasal, bronchial or oral route of administration of SIT (1, 2, 11). On the other hand, the sublingual-swallowed administration of SIT in higher doses, which is another type of local immunotherapy, has an A level evidence-based medicine for seasonal and perennial allergic rhinitis in adults and seasonal allergic rhinitis in children (1, 11, 12).

The clinical efficacy was demonstrated by DBPC study and later, with the increment in the allergen doses, the immunological changes were confirmed (12, 13). Recent papers have confirmed the long-lasting (14) clinical efficacy of sublingual administration in a randomised, placebo-controlled, double blind, double-dummy study (15).

In spite of the widespread knowledge in the scientific literature, the mechanism by which SIT achieves clinical improvement remains unclear. For subcutaneous immunotherapy the immunomodulation of the T-cell response is now accepted: immune deviation (increase in Th0/Th1), T-cell anergy (decrease in Th2/Th0), or both mechanisms (1). However, this is not so linear, because SIT did not induce Th1-type cytokine-associated pathology in treated respiratory allergic patients to aeroallergens (16). There are other

immunological changes with subcutaneous SIT: the rise in allergen-blocking IgG, the induction of IgE-modulating CD8+ T cells, the reduction of mast cells and eosinophils on the target mucosa, the decrease in inflammatory mediators (1, 5, 17-21) and a modulation on the inflammatory trafficking cells by reduced expression on VCAM-1 (22, 23).

Immune tolerance depends on different mechanisms, including T-cell anergy, T-cell depletion by apoptosis, and active immune suppression (24). One of the target mechanisms of SIT is the induction of tolerance to allergens to which the patient is sensitised (25). IL-10 is probably a relevant cytokine induced by this treatment and is related to regulatory T cells that actively control or suppress the function of others cells, generally in an inhibitory pattern (5, 25). The changes in microenvironment due to the decrease in histamine and PGE2 mast cell release and the IL-10 and TGF- β release by dendritic cells could deviate the T-cell population pathway in order to induce T-regs and finally a therapeutic tolerance (26). This recent findings agree with what is currently known about the regulation induced by SIT. It is not credible that it could promote a Th2 / Th1 switch (25).

Much of the knowledge on SIT is based on studies performed in subcutaneous route of administration, but increasing data are now available on sublingual vaccines. The absorption of allergen through the intact mucosa and the interaction with local dendritic cells could induce the process and immune-tolerance. Besides the intrinsic local effect, the swallowed allergen could induce an additional GALT-related mechanism (11, 12). In fact, the first method of sublingual administration of SIT in which the allergen was spat out is significantly less effective. Most of the mechanisms and immunological changes that occur with sublingual administration of high allergen dose have been recently demonstrated (1, 27-31).

There is lots of information available on immunomodulation with SIT, but studies on kinetics of SIT administration and its immune effects on allergic patients are still lacking.

The study of M Bagnasco *et al.* (32) was highly important in understanding much of the data. In this study, the administration of radiolabelled *Par-j-1* by ^{125}I isotope to healthy individuals demonstrated the local persistence of the allergen at the sublingual region for a long period of time. The systemic radioactivity was only present after swallowing the allergen. For sublingual SIT it seems that the allergen is degraded in the gastrointestinal environment and only peptides are absorbed (33). There are no similar studies in the literature concerning the subcutaneous route of administration.

Radiolabelling of blood cells is a nuclear medicine technique used in the study of inflammatory diseases such as chronic bowel inflammatory disease (34-37). We considered that this kind of methodology could be

interesting worth investigating further with regards to an *in vivo* approach to the study of SIT mechanism of action and immune response. We expected to study the migration of autologous peripheral labelled-leukocytes that were reinjected to the site of the allergen extract administration in allergic patients, and the other possible resulting events. We think that the biological response to the therapy can be easily observed. The site or sites where the immune response happen and the different times of the response, can be established. We decided to study the kinetic response to SIT in patients with different types of allergy concerning the severity, the type of allergenic extracts administered and different routes of administration.

MATERIAL AND METHODS

The Coimbra University Hospitals Ethics Committee and Research Commission approved the design of this study protocol and all the volunteer patients signed an informed consent form according to the international ethics consensus.

Subjects

13 adult allergic volunteer patients aged 20 to 51 year old were enrolled for study. All of them were submitted to SIT for at least 2 years and in all of them the clinical efficacy was demonstrated by complete remission of symptoms, the absence of medication with regular anti-allergic drugs, reduction of skin reactivity to allergens and reduction of serum specific IgE. In patients with previous anaphylaxis, the absence of allergic reactivity after the beginning of treatment was confirmed by challenge tests or immunoblotting studies.

The patients were selected according to the different allergenic extracts and routes of administration of SIT, including:

- > Aqueous subcutaneous allergenic extract (for anaphylaxis):
 - 2 patients - hymenoptera venom, honeybee venom extract;
 - 3 patients - latex extract.
- > Depot subcutaneous allergenic extract (for respiratory allergy):
 - 2 patients - *Dermatophagoides pteronyssinus* extract;
 - 2 patients - grass pollen extract.
- > Modified subcutaneous allergenic extract (for respiratory allergy):
 - 1 patient - grass pollen extract;
 - 1 patient - *Parietaria judaica* extract.
- > Sublingual allergenic extract (for respiratory allergy):
 - 2 patients - *Dermatophagoides pteronyssinus* extract.

■ *Dermatophagoides pteronyssinus* allergic patients (bronchial asthma and rhinitis) not submitted to SIT were studied as a control group.

None of the patients had other clinical disorders, namely inflammatory diseases. All the female patients were submitted to a pregnancy test on the day of the study to exclude pregnancy.

All the studies were performed under strict hospital medical care surveillance, in the day of SIT administration, according to each individual schedule of treatment.

Methods

■ **Technetium-^{99m} hexamethylpropyleneamineoxime leukocyte labelling (99mTc-HMPAO):** The white blood cells labelling process with 99mTc-HMPAO was performed according to the standard University Hospital Nuclear Medicine Department protocol, based on the technique described by Peters *et al.* (34). 42 ml of the patient's blood was collected from peripheral vein with a 60 ml plastic syringe, containing 6 ml of acid-citrate-dextrose (ACD) plus 6 ml of hydroxy-ethyl-starch (HES) at 6%.

The ACD anticoagulant prevents cells from sticking to the plastic wall of tubes and the HES accelerates erythrocyte sedimentation by affecting the charge of the sialic acid groups on the outer membrane of the red blood cell. This blood in the syringe was maintained in a vertical position for 30 mins in order to allow erythrocyte sedimentation. A second 10 ml syringe of blood was sampled with 1ml of ACD. This blood was centrifuged at 2 000 g for 10 min in order to obtain cell-free plasma (CFP).

After 30 mins of blood syringe sedimentation, the supernatant was centrifuged at 150 g for 5 min and the supernatant, platelet rich plasma (PRP) was discharged. The leukocyte pellet was resuspended in 0.6 ml of cell free plasma and labeled with 99mTc-HMPAO (Cereteq®, Amersham, UK). For the leukocyte labeling procedure, the HMPAO must be labeled first. For this purpose, a 1110 MBq in 5 ml of saline of 99mTc freshly eluted was added to a kit of HMPAO that contained 0.5 mg of exametazime, 7.6 mg of SnCl₂ and 4.5 mg of NaCl according to the manufacturer's indications. 3 ml of this 99mTc-HMPAO was added to the leukocytes resuspended in cell free plasma, and the mixture was maintained at room temperature for incubation, for 10 min, in order to label the leukocytes. After this time, 10 ml of PRP was added to the leukocytes in order to stop the labeling procedure, and the mixture was centrifuged at 150g for 5 min.

After centrifugation, the supernatant was removed and discharged and the labeled leukocyte pellet was resuspended in 5 ml of CFP, counted and re-injected into the patients.

The labeling yield ranged between 50% and 75%, as normally described.

The effective dose-equivalent for this examination was 0.021 mSv/MBq (38, 39).

■ **Allergenic therapeutic extract:** The allergenic extracts administered were from ALK-Abelló (Spain) for all the patients except for modified subcutaneous allergenic extract. These extracts were glutaraldehyde modified grass pollen (Bial/Aristegui- Spain) and depigmented *Parietaria judaica* pollen (Leti- Spain). All the patients were submitted to the maintenance dose according to the individual schedule.

One allergic patient of the control group was submitted to a 0.5cc subcutaneous phenol aqueous injection supplied by ALK-Abelló (Spain), and the other patient was submitted to a 0.5cc subcutaneous injection of a bacterial extract (Ribomunyl™, Pierre Fabre Médicament, France: ribosomal fractions of *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and the membrane fraction of *Klebsiella pneumoniae*), a nonspecific immunostimulant agent of Ig G production.

■ **Administration procedures:** Patients were lying supine under the gamma camera. A humeral vein was catheterised to permit the reinjection of labeled autologous leukocytes. The allergen extract was administered subcutaneously, according to the usual method, in the contra lateral arm. Both injections were given at the same time, simultaneously with the scintigraphic acquisitions.

The 2 allergic patients under sublingual SIT were also lying supine under the gamma camera at the beginning of the study. The drops of the allergenic extract were administered at the same time as the reinjection of the labeled autologous leukocytes in the humeral vein. Three minutes later, the patients swallowed the allergen extract.

For the control group the same procedure was adopted. The study was performed under the close supervision of allergist and nuclear medicine specialists.

■ **Scintigraphic acquisitions** (table 1): Scintigraphic studies were performed on a large field of view digital gamma camera (GE XR, Milwaukee, USA, interfa-

ced with a Camstar acquisition unit and an eNTEGRA processing unit), equipped with a parallel low-energy general purpose collimator.

A dynamic acquisition was obtained in anterior view at thoracic and neck level for 64x64 matrix for 60 minutes (120 frames x 30 seconds) followed by static images at 60, 90, 120, 180, 240, 300 and 360 mins after administration of the radiolabeled leukocytes and the allergen for 256x256 matrix and for 5 mins each. These static images were obtained in anterior and posterior views at thoracic level and anterior views at abdominal projection.

During the acquisitions, the visualized area was kept close to the gamma-camera collimator, and the patients were not allowed to move any part of the body, in order to maintain the geometry of the projections.

Throughout the acquisitions, the erythema and the wheal at the site of SIT administration were controlled. For quantitative results, the procedure was different according to the type of image. For the dynamic thoracic images, regions of interest (ROIs) were drawn at SIT administration, background area (muscle), and for possible focalisations (cervical and axillary areas and thorax). For the thoracic static views, an image corresponding to the geometric mean between anterior and posterior views was calculated, and ROIs were drawn at thoracic locations. For the static anterior abdominal views, ROIs were drawn over each location.

For each ROI the total counts, average counts per pixel and maximum counts were obtained, and decayed was corrected. With these values, uptake coefficients were calculated by the ratio between the maximum count rate at the target ROI and average counts at the background ROI. In order to normalise and to help with the interpretation of the results an uptake coefficient of the background ROI was calculated and subtracted from the uptake coefficient of the target ROIs.

■ **Data analysis:** The clinical pattern, allergen extract and route of administration were analysed individually and the different groups of patients were compared.

The statistical analysis was performed by descriptive analysis.

maximum count	average count
maximum count	average count background
Uptake Coefficient =	max count ROI /average count background
Uptake Coefficient =	max count /average count Uptake Coefficient corrected UCROI - UC BK

Table 1.

Results

There were no adverse events caused by radiolabeled autologous cells reinjection or allergenic therapeutic extract administration.

Clinical and allergenic therapeutic extract aspects are shown in table 2 and 3.

After scintigraphic processing, the ROI value was obtained at the site of SIT administration, thoracic, axillary and cervical area, abdominal focalizations and ROI background. We also calculated the corrected uptake coefficient (maximum count at the site of SIT ROI over average counts at the background ROI) at different focalisations at different times.

The uptake coefficients for the different focalisations on analysis are shown in Figs. 1-5.

We did not consider the scintigraphic acquisitions at the site of the allergenic injection (patient 1, 2 and 9) and the axillary area (patient 1) due to technical problems.

The inflammatory activity started early in patients treated with aqueous allergenic extract for anaphylaxis than for in patients treated with depot extracts

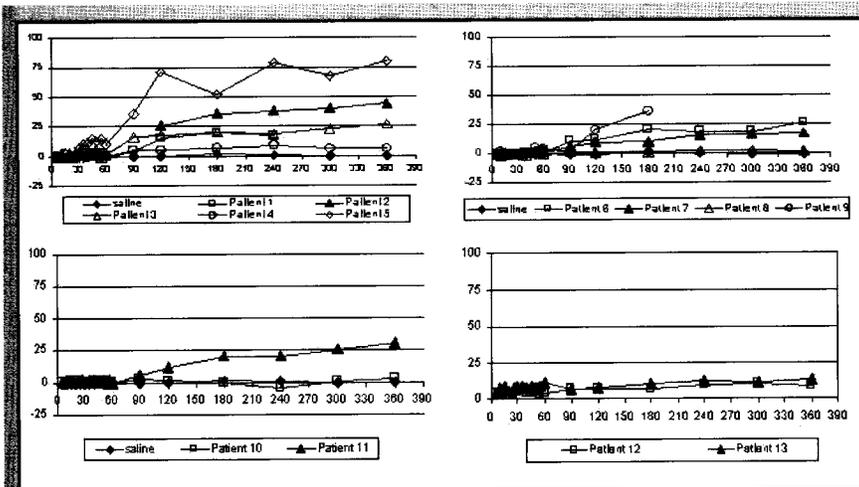
for other diseases (latex: 20-35 mins; hymenoptera venom: 15 mins; grass pollen: 25 and 30 mins; mites: 25 and 45 mins).

For sublingual allergenic extracts the inflammatory activity started promptly (at 5 mins) with a rapid increase that persisted during the study.

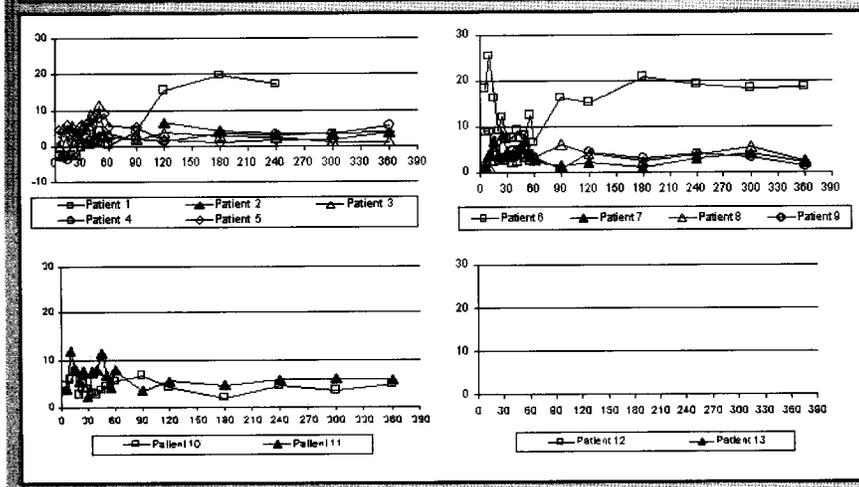
In patients submitted to allergoid, the inflammatory activity at site of administration of the extract started in the first minutes, although with a different pattern. In the allergic patient submitted to saline subcutaneous injection, we observed low values of activity at the site of administration, probably related with an irritative effect caused by phenol used as preservative. This observation confirms the sensitivity of the laboratory procedure. No other focalisations were observed during the study.

The control patient submitted to bacterial injection showed inflammatory activity at 10 minutes with an increase that was time related. We also observed activity in the axillary, the cervical and the thoracic areas, but with no focalisations at the bowel region, Fig. 6.

The inflammatory activity at the axillary and the cervical areas was present from the beginning of the



Figures 1: Uptake coefficient corrected of allergenic extract local administration.



Figures 2: Uptake coefficient corrected of axillary area focalisations.

Patient	Sex	Age (y)	Disease Evolution (y)	Disease	Allergen	SIT route	SIT extract	SIT years	SIT (cc)	SIT dose	Administered activity
1	F	34	7	A	L	sc		6	0.35	0.35	272.0
2	F	39	6	A	L	sc		5	0.35	0.35	240.5
3	F	28	7	A	L	sc		5	0.35	0.35	149.6
4	M	49	5	A	HV	sc		4	1.0	100	67.7
5	F	37	4	A	HV	sc		4	1.0	100	316.3
6	F	53	14	BA+AR	GP	sc	depot	4	0.60	1.5	337.2
7	F	24	10	BA+AR	GP	sc	depot	4	0.60	1.5	255.5
8	M	22	12	BA+AR	Dp	sc	depot	4	0.60	3.6	91.8
9	F	51	30	BA+AR	Dp	sc	depot	5	0.60	3.6	170.3
10	M	33	28	BA+AR	GP	sc	allergoide	5	0.70	36.72	476.7
11	F	49	34	BA+AR	Pj	sc	allergoide	3	0.50	12.0	132.7
12	M	20	10	BA+AR	Dp	sl	aqueous	3	5*	1.2	126.3
13	F	27	19	BA+AR	Dp	sl	aqueous	3	5*	1.2	254.6

Table 2: Clinical aspects of patients sample.

Disease: A= anaphylaxis; BA+AR= bronchial asthma+allergic rhinitis.

Allergen: L=latex; HV= hymenoptera venom (honeybee); GP= grass pollen; Dp= Dermatophagoides pteronyssinus; Pj= Parietaria judaica

SIT route: sc= subcutaneous; sl= sublingual

SIT extract: depot= aluminium hydroxide-adsorbed; allergoide=

SIT (cc): volume in ml administrated of the maintenance dose. Patients 12 and 13 are submitted to 5 drops

SIT dose: quantity of allergen in µg protein

Administered activity: MBq

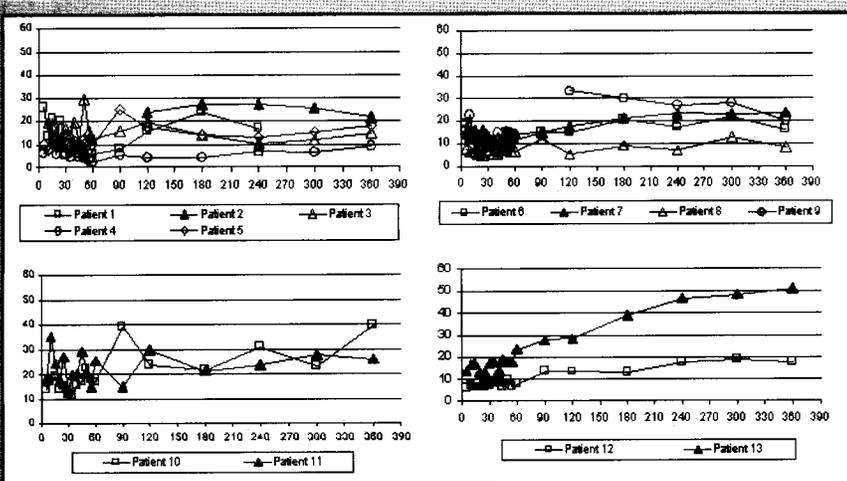
Controls	Sex	Age (y)	Disease Evolution (y)	Disease	Allergen	Injection	Volume(cc)	Administered activity
1	F	41	20	BA+AR	Dp	saline	0.50	306.4
2	F	45	20	BA+AR	Dp	bacterial	0.50	126.0

Table 3: Control group. Clinical aspects

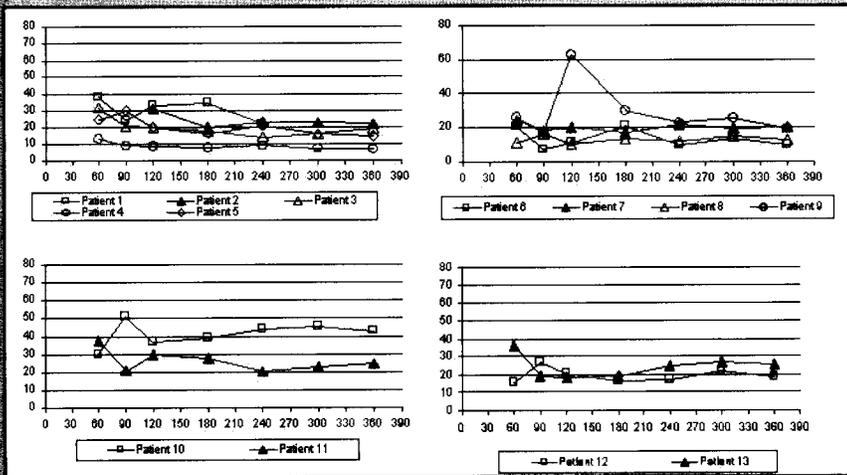
Disease: BA+AR= bronchial asthma+allergic rhinitis.

Allergen: Dp= Dermatophagoides pteronyssinus

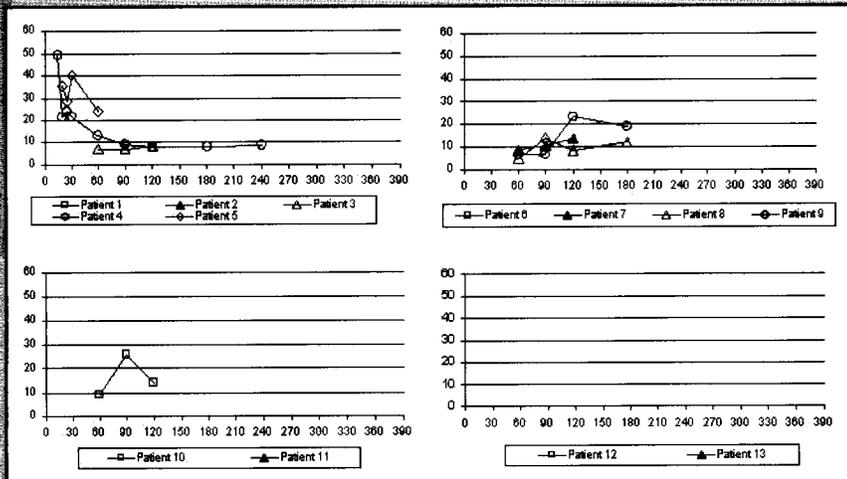
Administered activity: MBq



Figures 3: Uptake coefficient corrected of cervical area focalisations.



Figures 4: Uptake coefficient corrected of lung focalisations.

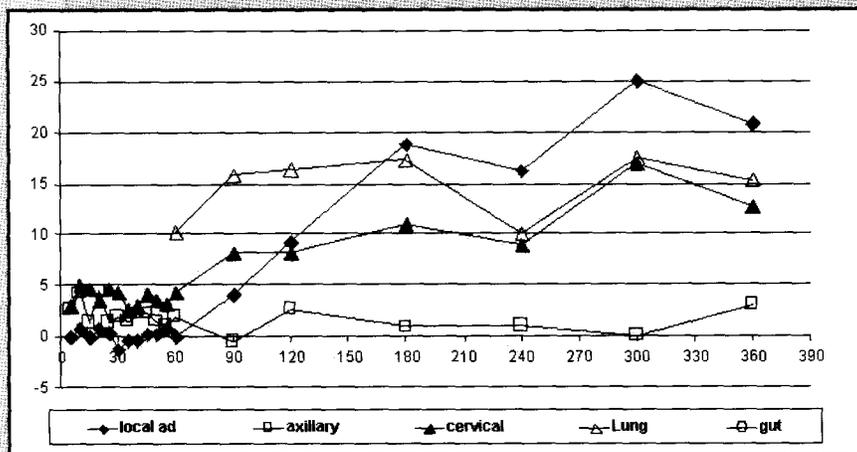


Figures 5: Uptake coefficient corrected of gut focalisations.

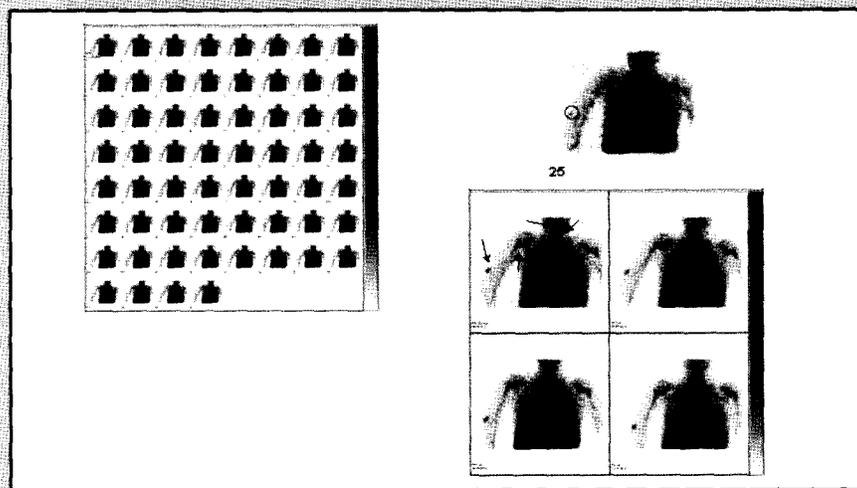
acquisitions for the subcutaneous allergenic extracts. There was a stabilization of the uptake coefficient values at 180 minutes. There were no focalisations at the axillary areas after sublingual immunotherapy. The high-labelled cell circulating pool could affect the interpretation of focalisations in the lung area in the first minutes, so we decided to calculate ROIs only

after 60 minutes after the injection, even though they were observed earlier. There were no significant differences among the different groups.

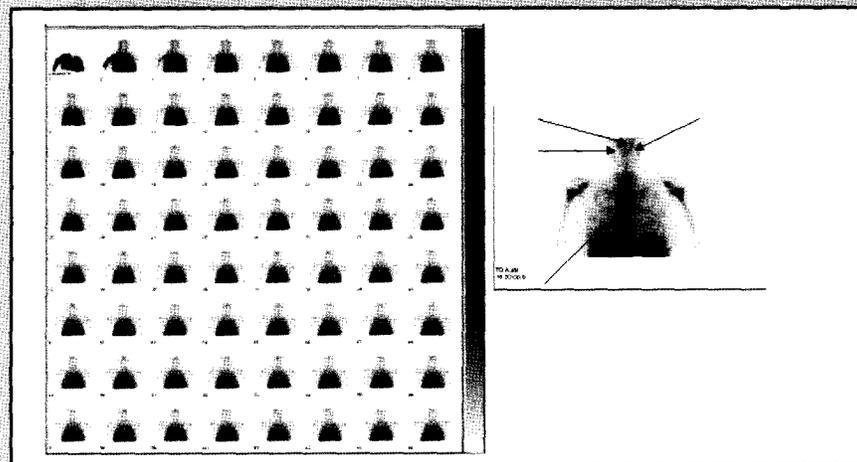
Focalisations at the abdomen acquisition views were observed in all the patients submitted to subcutaneous SIT. We only considered ROIs till 240 minutes. This time was coincident with the beginning of hepa-



Figures 6: Uptake coefficient corrected/time for the ROIs of the active control patient submitted to bacterial extract.



Figures 7: Patient 1 study, dynamic and static acquisitions. Inflammatory activity at 25, 90, 120, 180, 240 min.).



Figures 8: Patient 12 study, dynamic and static acquisitions. Inflammatory activity at 240 min.

tic/gall bladder excretion of the product. No bowel activity was observed in patients on SLIT treatment. There were other focalisations such as suprasternum and anterior mediastinum with high inflammatory activity, but we decided not to consider these areas because they were sites of vascular confluence. Bone marrow was another site of intense activi-

ty, higher than that usually observed by this technique. Distinct ROI count profiles were obtained with the different routes of administration and with the different allergenic extracts but all of them induced a time-dependent global inflammatory activation at sites related with the immune system (Fig. 7-10).

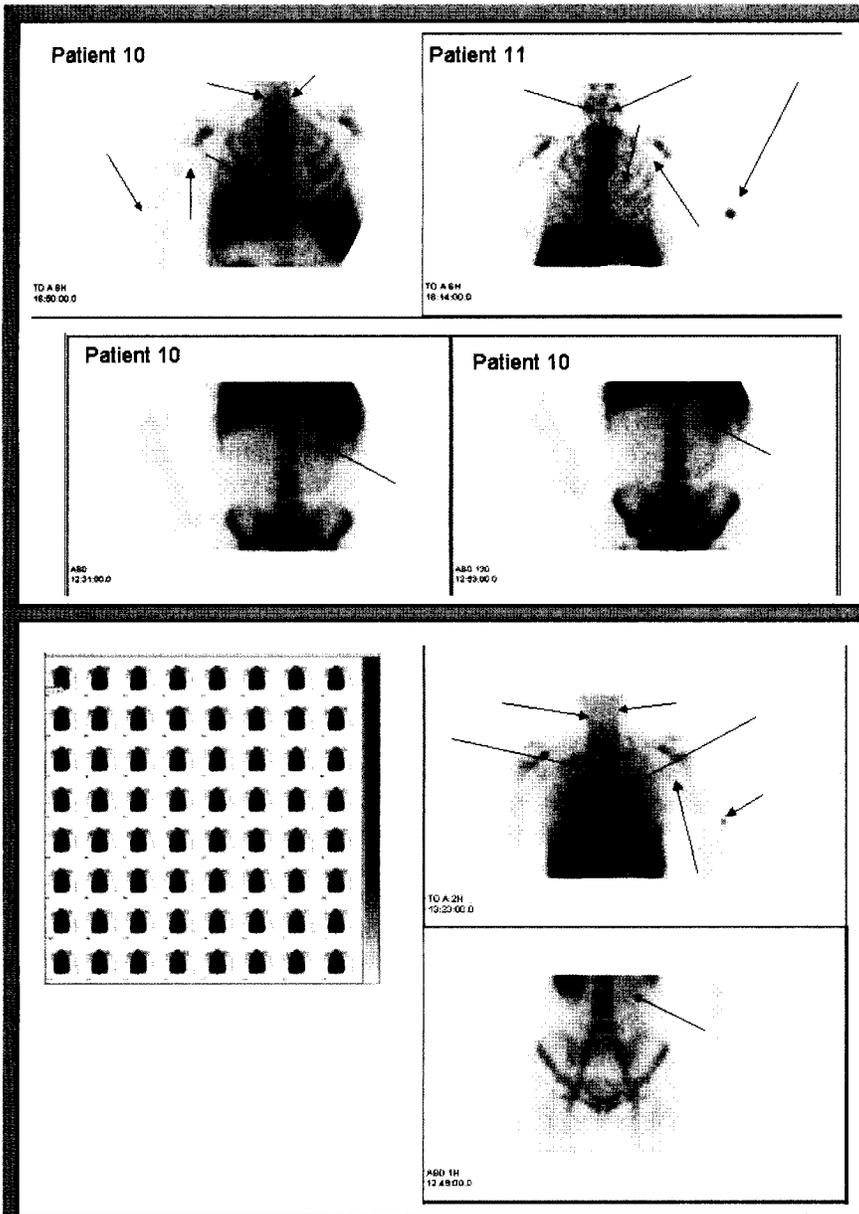


Figure 2: Static acquisitions in patients submitted to allergoid SIT.

Figure 10: Patient 7 study, dynamic and static acquisitions.

Concerning depot allergenic extracts, we did not observe differences between pollen and mite allergic patients.

For mite allergic patients, there were the same obvious differences concerning SIT routes of extract administration. The sublingual immunotherapy induced an early potent activity with higher uptake coefficient ROI values than the depot extracts, at site of the allergic extract administration.

The inflammatory activity at the local of SIT administration was greater than the erythema area involving the deeper layers and persisted even after local inflammatory signs disappeared.

DISCUSSION

In this study we decided not to label the allergen used, because it was only possible to make a linkage between an allergenic epitope and the radiolabel-drug, excluding the other epitopes. The decision to use the common administration of SIT seemed to be the procedure that minimised the error factors in the understanding of this therapy dynamics. The technique is now a routine procedure for analogous inflammatory pathologies. The risks are minimal, the radioactive burden is low, and the body-absorbed dose (effective dose) corresponds to about 3mSv, which is similar to one year of natural exposure in the continental area of Portugal.

Our first attempt was to study the local inflammation related to SIT administration. The activity observed in other areas and the speed of occurrence was a surprising result. Even in patients with excellent clinical response to immunotherapy, the administration of specific allergen resulted in a local inflammatory response that ranged from minimal intensity to erythema and/or wheal. The intensity of inflammation was higher for aqueous extract administrations in patients with the most severe allergic diseases. However, it was not related with clinical signs of inflammation at the site of administration. The allergoids were related with lower inflammatory activity when compared with depot extracts, as expected, according to the clinical knowledge (40). In the 2 patients submitted to sublingual extracts the beginning of the radioactivity was faster, with no signs of local effects. There was a minimal activity in the control patient who was submitted to phenol-saline injection, which is usually used to dilute the extracts. We considered that this reaction was related to phenol, which is a nonspecific irritant used as a preservative. This observation confirmed the sensitivity of the technique. The other control patient that was submitted to an injection of bacterial derived fragments, also revealed activity according to the expected IgG dependent mechanism of action of this immune-stimulant (41).

In the first minutes ascending drainage directed to the homolateral axillary area was induced by subcutaneous SIT. Cervical and superior mediastinum lymphoid tissues were the next structures involved. The late static acquisitions showed global activation on focalisations related to the immune system: lymphoid tissue neck/cervical, suprasternum, mediastinum, lung, gut and bone marrow. The early involvement of the central lymphoid structures was an unexpected finding. The maximum ROI values were increased even when the radionuclide was eliminated through urine and the half time was reached. This could reflect the magnitude of the therapeutical allergen mechanism of restimulation and immune-tolerance.

Concerning the sublingual immunotherapy the activity was reached faster than for subcutaneous SIT. There was an obvious difference in the inflammatory activity profile comparing these two types of immunotherapy. The most intense ROI values were achieved at the cervical lymphoid tissue, as expected. There was no activity in the axillar and the abdominal area, compared with depot extract. This data could explain the need for daily or three times a week administration of sublingual SIT in order to promote frequent stimulation of the immune system.

The allergoid extracts also induced inflammatory activity when compared with depot pollen extracts. There were obvious differences in local activity between both. Our results could deflect some criticism of lot of

authors who reject the use of polymerised vaccines or chemically modified allergens.

This technique did not allow labelling specific circulating peripheral cells. The pellet resulting from a blood sample contains all the lineages of white blood cells. At first, the lymphocytes seemed to be the only cells responsible for the local and global events in the study. However, we must reflect on all the knowledge about SIT, inflammation and immune response available till now.

Dendritic cells (DC) have been a matter of exhaustive study and seem to be a nuclear target of inflammation and immune tolerance. The professional APC are divided into two major systems: DC, including blood and tissue DC as well as epidermal Langerhans cells (LC), and the monocyte/macrophage system (42). Concerning DC there are two different subsets: DC1 or myeloid DC, and DC2 or lymphoid/plasmacytoid DC (42, 43). DC2 are mainly found in thymic medulla, tonsils and in T cell areas of secondary lymphoid organs, whereas DC1 are located outside the T cell areas. DC2 are unable to internalise foreign antigen (42). On the other hand, DC1 could traffic from the blood to the peripheral tissue to capture foreign antigens, may migrate to the draining lymphoid organs in order to prime T cells towards Th1 or Th2 cytokine profile (26, 43, 44). Under appropriate stimulus peripheral blood monocytes could differentiate to DC1 (42).

Langerhans cells belong to the DC1 system and are relevant in the skin. After local inflammatory stimulus the monocyte-chemoattractant protein (MCP) induces recruitment of LC progenitors from the bone marrow, migration to the skin and in the skin, and migration to peripheral lymph nodes (43). It is now well established that IL-10 DC release is an important factor of immune tolerance promoting Tregs lymphocytes (25, 26, 45).

The DC family of oral mucosa is different from that of the skin LC family. The oral microenvironment and TGF- β are relevant as well as IL-10 (43). They also presented increased expression of Fc ϵ RI on their surface, which was partially occupied by IgE molecules (43). The linkage of allergens to the Fc ϵ RI receptor contributes to tolerance by an IL-10 dependent mechanism (42, 43).

According to our results these data could be explained by the following hypothesis: The subcutaneous allergenic extract induced activation of LC, recruitment of other circulating DC/monocytes; migration to lymphoid areas; cooperation with DC2 and finally Tregs induced by IL-10 and tolerance towards sensitised allergens. The systemic activation of areas related with the immune system could support the global immunomodulation of SIT.

Large amounts of allergen are administered by sublingual SIT route in an area with close proximity to lymphoid organs such as the lingual amygdale. It is possible that DC could initiate the process as occurs

in the skin. However the presence of lymph nodes with other populations of DC (DC2) could lead to a saturation of activity of DC1 and the inability to cause an intense systemic inflammatory activity. The induced tolerance and the efficacy of this therapy are, consequently less intense and require frequent stimulation and higher allergen doses. On the other hand, the swallowed allergen could interact with gastrointestinal mucosa and promote additional tolerance mechanism, mediated by TGF- β and Th3 regulatory cells (43, 45).

Our results showed a real systemic effect of SIT, related to focalisations on immune system dependence. The inflammatory activity began quicker than we expected and there were obvious differences concerning the different extracts. There seems to exist an obvious difference between the kinetic of sublingual and subcutaneous routes of administration. Aqueous allergenic extracts induce more intense and global systemic inflammatory activity than depot extracts and ultimately the allergoid extracts.

In the future it will be important to study the kinetic/dynamic of the built-phase of SIT in animal models in order to understand how the tolerance/immunomodulation process occur throughout the treatment.

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AVOIDING PEANUTS MAY RESULT IN RECURRENCE OF ALLERGY, FROM THE JOURNAL OF ALLERGY & CLINICAL IMMUNOLOGY

MILWAUKEE — Children who keep avoiding peanuts after they outgrow their peanut allergy have a higher risk that their peanut allergy will return, according to a study in the November 2004 *Journal of Allergy & Clinical Immunology* (JACI). The JACI is the peer-reviewed journal of the American Academy of Allergy, Asthma & Immunology (AAAAI).

In their study, David M. Fliescher, MD, Johns Hopkins University School of Medicine, and colleagues studied 30 patients who had previously outgrown their peanut allergy. They sought to determine the percentage of patients who reacquire peanut allergy later; identify any risk factors for recurrent peanut allergy; and develop specific recommendations for treating patients who have outgrown peanut allergy.

Based on questionnaire results, repeat peanut-specific IgE levels, and repeat food challenges, the researchers found that patients who have outgrown their peanut allergy have approximately an 8% chance of it recurring. The study found this risk is significantly higher in patients who continue to avoid eating peanut after their allergy is resolved.

Based on the study's results, researchers recommend that patients eat concentrated forms of peanut at least once a month after outgrowing their peanut allergy in an attempt to maintain their tolerance to peanut.

Researchers also recommended that patients and families who rarely eat peanuts or eat them in limited amounts after outgrowing their allergy should continue to carry epinephrine at all times.

The AAAAI is the largest professional medical specialty organization in the United States representing allergists, asthma specialists, clinical immunologists, allied health professionals and others with a special interest in the research and treatment of allergic disease. Established in 1943, the AAAAI has nearly 6,000 members in the United States, Canada and 65 other countries. The AAAAI serves as an advocate to the public by providing educational information through its Web site at www.aaaai.org or the toll-free physician referral and information line at 1(800) 822-2762.

EDITOR'S NOTE: This study was published in the peer-reviewed, scientific journal of the American Academy of Allergy, Asthma & Immunology, but does not necessarily reflect the policies or the opinions of the Academy. To receive a copy of the study, please contact John Gardner (jgardner@aaaai.org) at (414) 272-6071. For more information and access to the archive of past JACI news releases, visit the Media Center of the AAAAI Web site, www.aaaai.org.