Enzyme Potentiated Desensitisation (EPD)
A promising low-dose method of immunotherapy

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EPD is a novel method of immunotherapy which was first used clinically in 1966. The method has a long record of safety and efficacy. More than 300,000 doses have been given without serious complications. EPD greatly expands the range of sensitivities and allergic syndromes which may be treated by immunotherapy.

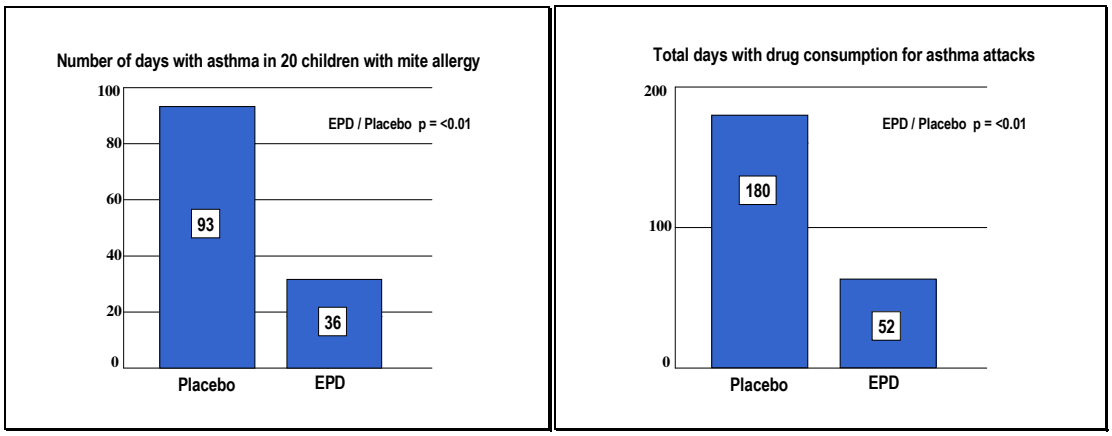
In 1966 it was recognised that the desensitising effect of a low dose of allergen could be potentiated by adding β glucuronidase. This gave rise to enzyme potentiated desensitisation (EPD). Nevertheless at first the effects were unpredictable. Further study showed that the immunological effect of the enzyme is controlled by a small dose of a sugar or a 1,3 diol. Increasing the dose of diol can switch the effect from hyposensitisation to hypersensitisation. There is a complex W-shaped dose-response curve (1). The dose of allergen is also important; large doses hypersensitise.

Stabilisation of the enzyme with protamine and optimisation of the doses of diol and allergen led to a reliable method of immunotherapy. The potency of the method should be judged from the published double-blind, placebo-controlled (DBPC) trials which have shown that a single preseasonal dose of EPD gives significant protection from seasonal pollenosis (2, 3, 4, 5, 6, 7, 16, 18). One trial (19) using two preseasonal doses was negative. In two DBPC trials, children with house dust mite-induced asthma were strongly protected by EPD (7, 8). (Table 1)

Table 1: Trials of EPD for inhalant allergy.

Authors	**Allergen**	**DBPC**	**A**	**P**	**Symptoms** **p =**	**Symptom-free days** **p =**	**Use of drugs** **p =**
Fell & Brostoff	Grass	Yes	22	22	NS. †	NS. ‡	< 0.02
Di Stanislao et al	Grass	Yes	20	20	NS	< 0.005	< 0.05
Longo et al	Grass	Yes	9	7	< 0.001	< 0.001	NS
Astarita et al	Parietaria Grass	Yes	10	10 *	< 0.001	-	-
Angelini et al	Parietaria Olive	Yes	11	10	0.001	< 0.001	< 0.001
Businco et al	Dust mite	Yes	10	10	< 0.05	< 0.01	< 0.01
Caramia et al	Grass Dust mite	Yes	8 27	8 27	< 0.001 < 0.001	-	< 0.001 < 0.001
Di Stanislao et al.	Grass	Yes	10	10	< 0.01		< 0.01
Boscolo & Brivio.	Grass	Yes	10	10	Rhinorrhoea 120 vs. 251 days Asthma 43 vs. 131 days		123 vs 219 days
Radcliffe et al	Grass	Yes	85	91	No differences		
Totals :	**4 sensitivities**		**222**	**225**	**10 Trials 9 significant**		

A : Active treatment. P : Placebo.
* Excludes second control group treated with allergen alone who were not “blind”.
† Unlimited intranasal steroid aerosol. All subjects titrated themselves to “comfort”.
‡ 14-day observation period at peak of pollen season.
Boscolo & Brivio considered it unnecessary to quote the statistical significance of their results.



From : Louisa Businco et al. (1996) J. Invest. Allergol. Clin. Immunol. **6(4)** : 270 – 76 (Ref 8)

The negative result of the trial by Radcliffe et al. has not been explained. The symptom scores of actively treated and placebo groups were almost identical. There was no "trend". In addition, the active treatment produced an irritating skin response. EPD for atopic inhalant sensitivities normally produces quite large skin wheals but there is no irritation.

Astarita et al. included a group in their trial who received pollen allergen but no enzyme. They showed that the seasonal symptoms in this group were identical to the group treated with saline placebo. They also reported that the skin reactions to antigen without enzyme were accompanied by itching.

This background suggests that the β glucuronidase administered in Radcliffe's trial may have been immunologically inactivated. This might have occurred through temporary freezing or heating during storage. The trial materials were stored in a hospital pharmacy and not under the control of the triallists. The same batches of enzyme and antigen were also dispensed from the laboratory for normal clinical use and appeared to perform satisfactorily.

Non-atopic food allergy EPD is also effective for non-atopic food allergy and DBPC trials have been successful in food-sensitive children suffering from hyperkinetic syndrome or migraine (9, 10). An open trial in unselected children, measuring attention deficit by computer, has shown that management by EPD or methylphenindate are equally effective. (11)

Placebo-controlled double-blind trial of EPD for food allergy in childhood migraine

	Active EPD	Placebo	Total
Undecided	0	1	1
Left Study	2	4	6
Unsuccessful	**2**	**9**	11
Successful	**16**	**6**	22
Total subjects	20	20	40

Fisher's exact test : p = <0.01 Aktuelle Neuropadiatrie 1992 Lischka A., Bernett G. (Eds.) (Ref 9)

Placebo-controlled double-blind trial of EPD for food allergy in hyperkinetic children

	Active EPD	Placebo	Total
Undecided	2	1	3
Left Study	2	1	3
Unsuccessful	**0**	**14**	14
Successful	**16**	**4**	20
Total subjects	20	20	40

Fisher's exact test : p = <0.01 Lancet **339** : 1150 – 3 (1992) (Ref 10)

Mixed allergens It has been found that inclusion of multiple allergens in the EPD formulations protects against recruitment of new sensitivities and is considered best practice.

The successful trials of EPD for inhalant allergies have all employed the same comprehensive allergen mix. Similarly, the successful trials of EPD for childhood migraine and hyperkinetic syndrome used the same mixed food extract for all patients even though they had different food sensitivities. These are the first successful DBPC trials of any method of immunotherapy using a mixture of more than one allergen.

EPD employs a dose of β glucuronidase which is physiological. The normal plasma concentration of the enzyme is 15-90 Sigma units/ml. The dose contained in a 0.05 ml. intradermal injection of EPD is 20 Sigma units. The lysosomes of macrophages and polymorphs contain large quantities of β glucuronidase, much of which is released into the tissues during inflammation or allergy. Many years ago May and his colleagues investigated release of β glucuronidase as a possible *in vitro* blood test for allergy. Histamine release proved to be more specific for immediate-type allergy.

Safety The doses of allergen required for EPD are also extremely small. The highest doses, used for pollenosis and house dust mite-sensitive asthma, are equivalent to conventional skin prick tests. The lowest doses, used to treat many non-atopic sensitivities, are at least one million-fold less. The result is unusual safety compared with other methods of immunotherapy.

The highest doses of food extracts employed are physiological. After a meal, unaltered food proteins appear in the bloodstream at concentrations of 2.5-5 ng/ml. Food extracts are used for EPD at doses of 10 ng. or less. How the administration of physiological doses of enzyme and antigens in a small intradermal injection can alter the immune status of the whole body is not yet fully explained.

The ultra-low doses of activators and allergen extracts and the complex W-shaped dose-response relationships present in EPD have led some authorities to consider that the method obeys homoeopathic principles. Nevertheless, the high degree of efficacy demonstrated in 11 successful placebo-controlled double-blind clinical trials indicates a potent immunological mechanism which is much more “powerful” than a homoeopathic remedy. EPD is a growing point of conventional medicine. It is not “alternative”.

When used to treat simple inhalant allergies, EPD is much safer than conventional immunotherapy and can be used by allergists without extra training. The use of EPD to treat eczema, migraine, hyperkinetic disorder or other non-atopic allergy syndromes requires a specialist knowledge of each syndrome and the role of allergy. An expert knowledge of EPD is also needed.

Drug Treatment When desensitisation is effective, drug consumption usually decreases and may become unnecessary but at the start, EPD should be used in parallel with optimal conventional therapy including drugs. There is a literature suggesting that some drugs, including some used to treat allergy, interfere with the development of immune tolerance by lymphocytes and may favour allergic disease. There are ways in which these can be avoided at critical times to permit EPD to be effective.

Mechanism β glucuronidase is an active enzyme only at acid pH. At neutral pH it is an adhesion molecule concerned with interactions between resting T lymphocytes, keratinocytes in the skin and polysaccharides such as hyaluronic acid in the intercellular space (12, 13). In cultures of human lymphocytes the enzyme is a mitogen (14). Under normal circumstances β glucuronidase is likely to be a significant physiological up-regulator of the lymphocyte immune response.

CD4+ Th1 and CD4+ Th2 lymphocytes produce different cytokines (Th1**:** IL-2 and γ-interferon, Th2**:** IL-4 and IL-5). Atopic allergy involves an excessive Th2 lymphocyte response to allergen. The IL-4 (plus IL-13 from macrophages) switches IgM-producing B lymphocytes to make IgE. The IL-5 stimulates eosinophils. Large numbers of Th2 lymphocytes are found in the lungs of atopic asthmatics. In non-atopic “intrinsic” asthma the IgE is normal but similar numbers of Th2

lymphocytes are present in the lungs. CD8+ T lymphocytes are also present and the γ interferon they produce may prevent the expected B cell response to IL-4. These B cells produce IgG4,not IgE. Mast cells are stimulated to release histamine by direct contact with the membranes of activated lymphocytes.

Contact allergy in the skin depends on delayed, "tuberculin-type" allergy driven by Th1 lymphocytes. No antibody is involved. The γ-interferon produced by this kind of lymphocyte down-regulates IgE and atopic allergy.

Between 1985 and 1995 the mechanism of "conventional" injection Specific ImmunoTherapy (SIT) was investigated chiefly by identifying the Th1 and Th2 cytokine profiles of allergen-sensitive T lymphocytes. Durham et al. showed that SIT deviates the atopic response to allergen away from Th2 towards Th1 and delayed tuberculin-type hypersensitivity.

Regulatory Lymphocytes

Attention has switched to the role of IL10 which down-regulates T lymphocyte responses. Akdis & Blaser showed that this is released in response to allergen by regulatory T lymphocytes (T reg1 cells) in T cell cultures from atopic patients treated by SIT (20). As yet, no changes in circulating IL10 have been detected following the classical forms of immunotherapy.

It seems that although conventional immunotherapy injections may deviate the lymphocyte response to allergen from Th2 towards Th1, the production of allergen-sensitive Treg1 lymphocytes is far more important.

Angelini's group have suggested that EPD also works by deviating the lymphocyte response to allergen from Th2 to Th1 (See below) but this seems unlikely since Simon McEwen has shown that EPD significantly reduces experimental contact allergy to 2,4 dinitrofluorobenzene which is a Th1-type response (14). EPD appears to down-regulate both Th2 and Th1 responses to allergen.

Production of regulatory T lymphocytes may be the chief effect of EPD. A blind laboratory study of blood IL6 & IL10 before and after EPD (15) (Table 2), showed that both cytokines increased significantly 24hrs. after treatment. Subsequently the IL6 fell to normal levels but the IL10 was still raised after 15 days. It seems that EPD is unique in producing a measurable rise in circulating IL10.

Table 2

Plasma IL-6 & IL-10 (ng./ml.) in grass pollen-sensitive asthmatic and normal children before treatment, 24 hrs after EPD and 15 days later.

	Patients n = 17	Controls n = 17	**p =**
IL 6 Baseline	17.08 ± 8.09	5.84 ± 2.15	< 0.002
24 hours	20.54 ± 12.37	6.89 ± 4.20	< 0.005
15 days	10.64 ± 6.29	9.10 ± 4.27	NS.
IL 10 Baseline	112.46 ± 18.51	64.39 ± 10.15	< 0.005
24 hours	146.54 ± 26.31	53.65 ± 12.73	< 0.005
15 days	143.04 ± 12.57	66.87 ± 18.54	< 0.005

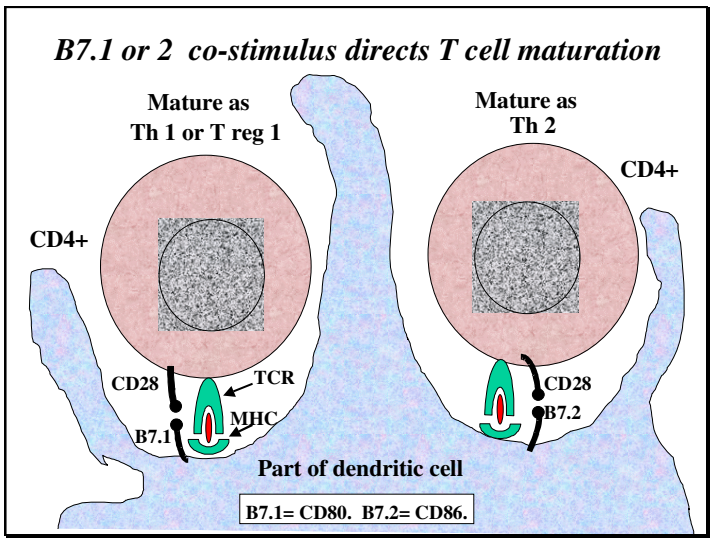
Ippoliti et al. 1997 (Ref 15)

Angelini's work

T lymphocytes are programmed by antigen-presenting cells (Dendritic cells, DC) which are particularly numerous in the dermis. These respond to antigens/allergens by migrating to the regional lymph nodes. There they present processed fragments of antigen ("epitopes") to lymphocytes via the MHC complex. By itself this stimulus would instruct a lymphocyte to become anergic to the antigen. (Although the individual lymphocyte would be tolerant, it would be incapable of any regulatory function.)

To develop an active response to antigen, a lymphocyte requires the extra stimulus from co-stimulatory ligands. B7 on the DC must link with CD28 on the lymphocyte. Variations of B7 can also direct the lymphocyte to mature down different pathways. B7.1 favours Th1 or T reg1. B7.2 favours Th2. (Fig 1).

Fig 1



Angelini's group conducted two DBPC trials of EPD for pollenosis. In the second (16) they again showed a significant difference in clinical scores between active and placebo groups. ($p = < 0.01$). They also generated DCs from monocyte precursors in peripheral blood samples (6 day's culture with GM-CSF & IL4 followed by lipopolysaccharide stimulation to mature).

DCs from the active treatment group expressed significantly less B7.2 ($p = < 0.05$). There was also less expression of CD83. IL10 was absent in DC cultures from treated subjects. (IL10 produced by DCs is thought to favour Th2 rather than tolerance). There was no difference in expression of MHC or B7.1. These results parallel the switch from Th2 to Th1 shown to result from conventional immunotherapy injections (SIT) but it seems likely that as a further parallel, the main effect of EPD, like SIT, is to generate a population of allergen-sensitive Treg1 lymphocytes. Nevertheless the real target of EPD may be an early stage of DC development.

Deviation of the immune response towards Th1 can not be the main mechanism of EPD. Delayed contact hypersensitivity in the skin is driven by Th1 lymphocytes and Simon McEwen has shown that EPD down-regulates experimental contact hypersensitivity. (14)

New methods of down-regulating allergic disease are being developed which produce tolerance by blocking various aspects of immune function such as IgE in a non-specific way (17). These are likely to involve risks due to the impairment of immune competence. In contrast, EPD depends on the use of an immune modulator which will increase the T lymphocyte response to any antigen which is not present at the correct ultra-low dose. EPD has a long track record and careful follow-up of patients has shown that the method is as safe as expected.

An Audit of EPD

The American EPD Society (AEPDS) studied EPD with Investigational Review Board approval using computer audit (Chief investigator Dr. W. A. Shrader). In September 1999 the number of patients assessed after at least 3 doses of EPD was 5,400. This is the first large-scale audit of any form of allergy immunotherapy. The results of this open study (Table 3) show that between 70% and 80% of patients with simple allergic conditions such as rhinitis, asthma and urticaria rated their improvements as “greater than 50%”. The unusual safety of EPD allowed a large number of patients suffering from immediate-type food allergy to be included in the study (Table 3). No severe reactions were reported.

This audit also illustrates the wider protective immunostimulant effect of EPD. Of 245 children suffering from repeated ear infections, 89% rated their improvement greater than 50%.

Table 3. Results of AEPDS audit of EPD to Sept 1999.

Patient's assessment of symptom severity on follow-up after 3 or more doses

	Total Patients	**50% improved**	**No better / Worse**
Rhinitis Seasonal	1297	79%	10%
Rhinitis Perennial	2274	73%	13%
Asthma Seasonal	214	77%	10%
Asthma Perennial	741	72%	13%
Urticaria	239	77%	11%
Food allergy: Immediate	508	73%	11%
Food allergy : Other	2802	72%	13%
Irritable bowel	592	71%	13%

A total of 58 symptoms were followed up.
27 symptoms scored >50% improvement in >65% of patients.

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