

Novel Delivery Routes for Allergy Immunotherapy

Intralymphatic, Epicutaneous, and Intradermal



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KEYWORDS

- Epicutaneous allergy immunotherapy • Transcutaneous allergy immunotherapy
- Respiratory allergy • Food allergy

KEY POINTS

- Subcutaneous immunotherapy deposits the allergen in the fat rather than stimulating the immune system. Therefore numerous injections are required to ameliorate symptoms.
- The ideal route for AIT contains dense APCs to enhance effects but few mast cells and blood vessels to reduce local and systemic side effects.
- Lymph nodes contain the highest density of APCs with only few mast cells and blood vessels, making ILIT highly efficient.
- Similar to mucosal epithelium, the epidermis has dense APCs with no mast cells or blood vessels. EPIT should therefore be as safe as SLIT.
- Allergen administered to the epidermis rapidly diffuses to the dermis. We expect diffusion toward blood vessels to be safer than injection into vascularized tissue.

INTRODUCTION

Today, up to 30% of the population in industrialized countries suffers from IgE-mediated allergies, which have therefore become an important socioeconomic burden. Pharmacotherapy with local and oral antihistamines and nasal corticosteroids ameliorates IgE-mediated symptoms efficiently,¹ but cannot stop progression of the causative immunologic imbalance, and therefore progression of rhinoconjunctivitis to asthma and to cross-reactive food allergies. The only disease-modifying treatment that also has a long-term effect is allergy immunotherapy (AIT).^{1,2} More than a century ago, Noon³ introduced subcutaneous allergen-specific immunotherapy (SCIT). However, despite its high efficacy and long-lasting symptom amelioration, less than 4% of allergy patients choose to undergo such AIT, mainly because it has two major

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disadvantages. First, AIT is time consuming because it requires 30 to 70 visits to a medical practice. Second, subcutaneous allergen injections are associated with local and systemic allergic side effects.^{4–6} Thinking generally, these two drawbacks may be addressed by the following strategies.

Measures to Reduce the Number of Allergen Administrations in Allergy Immunotherapy

To reduce the number of injections, immunogenicity of the allergen administration has to be enhanced, for instance by increasing the allergen dose. AIT has a clear dose effect,⁷ but allergic side effects strongly limit the dose that can be given. Making allergens hypoallergenic by chemical modification to allergoids,⁸ recombinant modification,⁹ or by using non-IgE-binding peptides^{10–12} may also permit increased allergen doses, but the modifications often negatively affect allergen immunogenicity. A reduction of injection numbers may also be achieved by replacing the classically used aluminum salts with a more T helper (Th) 1 promoting adjuvant, such as the Toll-like receptor (TLR) ligands CpG oligodeoxynucleotide¹³ or monophosphoryl lipid A, a detoxified version of lipopolysaccharide.^{14–16} The number of injections may also be reduced by changing to a more efficient route of allergen delivery. Ideally this route would be characterized by a high density of antigen-presenting cells (APCs). The latter are present at highest density in secondary lymphatic organs, such as lymph nodes, and indeed, when allergen is intralymphatically, the number of injections could be reduced to only three.^{17–20} Intralymphatic immunotherapy (ILIT) is discussed in detail later.

Measures to Improve Allergy Immunotherapy Safety

To improve safety of AIT, inadvertent allergen delivery to the blood vasculature must be avoided, ideally by delivery of the allergen to nonvascularized tissue. Sublingual immunotherapy (SLIT) fulfills this criterion, because allergen is delivered to the oral mucosa, which is covered by a multilayered epithelium. The allergen diffuses down into deeper mast cell containing layers, and this diffusion is responsible for the frequently observed local oral side effects.^{21,22} The layer below the epithelium also contains a high density of blood vessels. However, it seems that when there is no microtrauma to this vasculature, it rarely happens that significant amounts of allergen reach the circulation, for which reason SLIT has proved safe in terms of systemic allergic side effects.^{21,22} The same should hold true for epicutaneous allergy immunotherapy (EPIT), where allergen is administered to the nonvascularized epidermis. An advantage of EPIT over SLIT is that keratinocytes can additionally be activated by physical irritation, such as abrasion or adhesive tape stripping, or also by adding adjuvants.²³ Such epithelial irritation increases the expression of proinflammatory cytokines, such as interleukin (IL)-1 α , IL-6, and tumor necrosis factor- α , thus skewing the immune response toward Th1,²⁴ and also activating Langerhans cells (LCs). Therefore, EPIT may not only reduce side effects by minimizing the risk of allergen inadvertently reaching the blood vasculature, but also shorten treatment duration by increasing immunogenicity. We have also observed that epicutaneously administered allergen rapidly and efficiently diffuses down into the dermis.²⁵ Interestingly, another group has recently demonstrated that intradermal administration of even extremely low doses of allergen was able to induce tolerance.²⁶

INTRALYMPHATIC ALLERGEN-SPECIFIC IMMUNOTHERAPY

The concept that antigen localization is a key parameter that determines the strength of the immune response was pioneered in Zurich by the work of Rolf Zinkernagel,

Nobel laureate in Medicine. The concept is simple: because an immune response requires the interaction of three important immune cells (antigen-presenting dendritic cells [DCs], T cells, and B cells), a reaction is more likely to happen at a site where high numbers of these three cell types are present, that is, in lymphoid organs, such as lymph nodes, whereas antigens outside of these organs are largely ignored by the immune system.²⁷ A great number of preclinical and clinical studies have demonstrated the potency of intralymphatic administration of peptides, proteins, DNA, RNA, bacteria, viruses, or DCs, as comprehensively reviewed.^{19,28–31} Studies in mice have demonstrated that ILIT with bee venom allergens, food allergens, such as ovalbumin, and allergen extracts from grass pollen, birch pollen, and cat dander stimulates robust antiallergic and protective B- and T-cell immune responses.^{32–36} Compared with SCIT, ILIT enhanced efficiency of immunization, inducing allergen-specific IgG2a antibody responses 10 to 20 times higher with only 0.1% of the allergen dose.³⁴ Moreover, ILIT enhanced IL-2, interferon- γ , IL-4, and IL-10 secretion when compared with SCIT,³⁴ suggesting that ILIT did not polarize the response but generated overall stronger responses. The reason seems to be simple: ILIT delivers approximately 100-times more allergen to lymph nodes than any other route, as demonstrated in biodistribution studies after ILIT in mice³⁴ and humans.^{29,30} Because adverse side effects are related to the allergen dose, ILIT would be expected to have a lower incidence of adverse effects.

In a Swiss trial, 165 patients with grass pollen–induced rhinoconjunctivitis were randomized to receive either 54 SCIT injections with pollen extract over 3 years (cumulative allergen dose 4,031,540 subcutaneous units) or three ILIT injections over 2 months (totally 3000 subcutaneous units).¹⁷ Intralymphatic injections were also measured to less painful than venous punctures. Increased nasal tolerance was demonstrated in the ILIT group within 4 months of treatment. Tolerance was long lasting and comparable with SCIT-treated patients who received 3 years of treatment. ILIT ameliorated symptoms, enhanced compliance, reduced skin prick test reactivity, decreased specific serum IgE, and was associated with fewer adverse events than SCIT. Whereas we compared ILIT with SCIT with grass pollen extract, a Swedish study confirmed the positive results of ILIT in a double-blind placebo-controlled trial.³⁷ By the same token, in a randomized, placebo-controlled, and double-blinded trial, we demonstrated efficacy of ILIT with major histocompatibility complex class II–targeting cat dander allergen (MAT-Fel d 1).¹⁸ Three monthly injections with MAT-Fel d 1 improved nasal allergen tolerance and stimulated significant regulatory T-cell responses and IgG4 without any significant adverse events.

Time Interval Between Injections in Intralymphatic Immunotherapy

In a double-blind placebo-controlled clinical trial, Witten and colleagues³⁸ found that three or six intralymphatic grass pollen injections induced some desired immunologic changes, such as a regulatory T-cell response and elevated IgG4, but that there was no improvement of clinical parameters, such as symptom or medication scores; if anything, symptoms tended to worsen. The authors concluded that their data were conflicting with ours^{17,18} and that of Hylander and colleagues.²⁰ However, Witten and colleagues³⁸ used a different protocol. We and Hylander and colleagues²⁰ used a 4-week time interval between injections, whereas Witten and colleagues³⁸ used only 2 weeks. It is well known from vaccine immunology³⁹ that shortening the time interval from 4 to 2 weeks interferes with memory B-cell formation and affinity maturation, which both require phases where only small amounts of antigen are present in lymph follicles. Small amounts enable competition for the antigen, which again positively selects for high-affinity memory B cells. Similar effects are postulated for affinity

and functionality of the T-cell response.⁴⁰ As a recent example, Patel and colleagues¹² observed that four peptide injections (Cat-PAD) with monthly intervals successfully tolerized patients, whereas eight injections with 2-week intervals did not work. Furthermore, shortening the time interval between injections is known to polarize the immune response toward Th2,⁴¹ which may explain why Witten and colleagues³⁸ observed symptoms to worsen.

We are meanwhile aware of clinical trials being performed in several centers across the world, such as in Sweden, Norway, Denmark, China, South Korea, and the United States (Cardell LO, Hoffmann HJ, Weinfeld D, Patterson A, personal communication, 2015). In thin-spread societies, such as in Scandinavia, patients have to travel long distances to get immunotherapy, so that a reduction in the number of shots would also have a direct socioeconomic impact, such as less days off from school and work.

Another consideration that speaks for ILIT is that in SCIT and SLIT, nearly half of patients do not finish the whole treatment course.⁴² In our trials with ILIT, we observed that everybody who started treatment received all three injections and thus the full treatment course. In conclusion, AIT directly into a subcutaneous lymph node, instead of subcutaneous administration, is (1) practically painless, (2) readily feasible, (3) reduces the required allergen dose and therefore improves safety, (4) reduces the number of allergen injections to three, (5) reduces the treatment duration from 3 years to 2 months, and (6) enhances patient compliance.

ALLERGEN-SPECIFIC EPICUTANEOUS IMMUNOTHERAPY

The epicutaneous route of allergen administration is by no means new. In fact, as a route of vaccination it was used in ancient times, and Edward Jenner applied cow pox virus to scarified human skin. Forgotten for a long time, at the beginning of the twenty-first century epicutaneous vaccination had its second revival, driven by the increasing interest in novel needle-free vaccination routes. Epicutaneous vaccination against *Escherichia coli*-induced traveler's diarrhea made the first step.⁴³ Animal models have so far shown success against infection with *Helicobacter pylori*,⁴⁴ influenza virus,⁴⁵ and diphtheria toxin.⁴⁶ The protective mechanism in all of these applications relies on induction of humoral immunity dominated by IgG1 and IgA. Studies testing epicutaneous vaccination against human immunodeficiency virus also found induction of mucosal cytotoxic T cells together with secretion of mucosal antibodies.⁴⁷ Another field of application is cancer immunotherapy. Several studies revealed promising results with EPIT against skin cancer based on induction of potent CD8⁺ T-cell responses.^{48,49} Not only has EPIT been demonstrated to induce effector T-cell responses, but also suppressive T-cell responses when EPIT was used to inhibit experimental allergic encephalomyelitis.^{50,51}

History of Epicutaneous Immunotherapy in Allergy

EPIT as a treatment of allergies was introduced already in 1917 when Besredka showed that EPIT induced specific antibodies.⁵² The first case study on successful EPIT was reported in 1921 by Vallery-Radot and Hangenau,⁵³ who found that allergen administration onto scarified skin reduced systemic allergic symptoms in patients allergic to horses. A decade later, when the risk of suffering a "pollen shock" during AIT was recognized to be a considerable danger of subcutaneously administering allergen to highly sensitized patients, a method called intradermal allergen specific immunotherapy received much attention.^{54,55} Based on the observation that patients with hay fever occasionally experienced symptom amelioration after "intradermal pollen tests," Phillips⁵⁵ started to treat highly sensitive patients and patients requesting

coseasonal treatment by administration of pollen extract. Strikingly, such intradermal AIT proved to be safe and highly efficacious leading to symptom relief after administration of only three doses.⁵⁵ At the same time, Ramirez treated patients allergic to grass pollen with a method he called “cuti-vaccination,” which consisted of administration of pollen extract on scarified skin.⁵² Based on these results, it was suggested in the 1930s already that the subcutaneous route might not be optimal for AIT.⁵⁴

Between 1950 and 1960, French allergologists revisited EPIT.^{52,56,57} Pautrizel and coworkers⁵⁷ administered the allergen extract onto slightly rubbed epidermis. Even though the reported results were excellent, a large number of applications were necessary until symptom relief was observed. In contrast, Blamoutier and coworkers^{52,56} applied the allergen drops onto heavily scarified skin: “On the proximal volar aspect of the lower arm, in a square area of 4×4 cm, chessboard-like horizontal and vertical scratches are made with a needle [...] These scratches should be superficial and not cause bleeding.”⁵⁸ Consistently, allergic side effects were observed only rarely when allergen was applied via the skin and they were at all times milder than under conventional SCIT.^{52,55,57} These promising results were supported by several studies performed in the subsequent years all over Europe, from Switzerland to Portugal.^{58–60} Overall, symptom relief was obtained rapidly and allowed for coseasonal treatment. The reported treatment success rates of 80% exceeded the success rates under conventional SCIT.⁵⁸ Despite such successful results with the French *méthode de quadrillage cutané*, reports on this promising administration route disappeared into oblivion for almost half a century.

Epicutaneous Immunotherapy with Aeroallergens

Although there is strong scientific and historical evidence for EPIT in allergy, there existed no double-blind placebo-controlled clinical trials, a fact that led our group to revisit EPIT. Driven by the idea to find a patient-convenient application route of AIT, and based on the good accessibility of the skin and its high density of potent immune cells, our group performed three clinical trials to test efficacy and safety of EPIT. To keep epithelial barrier disruption minimal, we replaced skin scarification by adhesive tape stripping.⁶¹ Besides enhancing the penetration of allergens by removing the stratum corneum,⁶² repeated tape stripping also functions as a physical adjuvant through activation of keratinocytes, which then secrete various proinflammatory cytokines (IL-1, IL-6, IL-8, tumor necrosis factor- α , and interferon- γ) favoring maturation and emigration of DCs to the draining lymph nodes.^{63,64} The first pilot trial revealed that patients treated with a total of 12 patches containing grass pollen extract experienced significant alleviation of hay fever symptoms compared with placebo-treated patients. In line with the historical study results described previously, no severe systemic allergic reactions were reported. The only adverse events observed were very mild local eczematous reactions under the skin patch.⁶¹ When looking at all 12 patch applications, in 15 out of the 21 verum-treated patients, mild eczema was observed, whereas eczema was seen in only 5 out of the 15 placebo-treated patients. When looking at a single patch application, in roughly half of the verum-treated patients, eczema was observed under the patch, with a severity score between 3 and 6 on a scale ranging from 0 to 18. To exclude that the occurrence of local adverse effects might have partially unblinded the study, we analyzed whether the occurrence of eczema under the patch correlated with symptom amelioration, but could not find such correlation.

Encouraged by these results, a second phase I/IIa trial including a total of 132 patients with grass pollen allergy was initiated to find the optimal treatment dose of EPIT. Enrolled patients were treated with a total of six patches during the pollen season. We

found a clear positive correlation between the administered allergen dose and the clinical effect.⁶⁵ Also, dose-dependent local adverse effects were observed at the site of patch application. Pruritus was the most frequently reported adverse event, followed by eczema observed after patch removal. Interestingly, with every subsequent patch application, there was a reduction of local adverse events. After the sixth patch application, merely half as many local adverse events were reported. This reduction was not explicable by local depletion of immune cells or degranulation of mast cells, because each of the six patches was applied to a different area of the arm. Therefore, the reduction of local adverse events is likely to be explained by tolerance induction. A third clinical trial investigated the immunologic changes induced during EPIT and found an increase in allergen-specific IgG4.⁶⁶ Our results have meanwhile been confirmed by an independent group, demonstrating efficacy and safety of EPIT in children allergic to grass pollen. Hay fever symptoms and the use of antihistamines were significantly reduced in the active treatment group.⁶⁷

So far, there is no head-to-head comparison of EPIT with other routes of administration, except in mouse models. Using the major grass pollen allergen Phl p 5, in the mouse, EPIT was found to be equivalent or better than SLIT.⁶⁸ Although EPIT and SLIT induced similar IgG2a levels and also led to a similar reduction in IgE levels in sensitized mice, it was only EPIT that led to a significant reduction of eosinophil counts in the bronchoalveolar lavage in the asthma model. Also in mice, we have compared SCIT with EPIT using ovalbumin as the allergen.²³ Although EPIT without adjuvant was less immunogenic than SCIT, EPIT with an adjuvant was found to be more immunogenic, so that EPIT and SCIT seemed comparable in efficacy.

Epicutaneous Immunotherapy with Food Allergens

A clinical pilot trial to test clinical efficacy and safety of EPIT using the Viaskin EDS (DBV Technologies, Bagneux, France) in children suffering from cow's milk allergy showed a nonsignificant tendency toward increased cumulative tolerance doses after a 3-month treatment period.⁶⁹ Treatment was well tolerated with no systemic anaphylactic reactions, but a significant increase of local eczematous skin reactions was observed. Such good safety results are crucial especially when considering the use of EPIT as treatment option for food allergies, for which conventional SCIT is impractical because of an unacceptably high rate of anaphylactic reactions.⁷⁰ To substantiate these early findings and aiming to develop a definitive therapeutic option for food allergy patients, an extensive clinical trial program has been initiated with the objective to test treatment efficacy of EPIT with the Viaskin EDS in patients with peanut allergy. A multinational double-blind, placebo-controlled, randomized phase IIb trial has already generated positive clinical results.⁷¹

Methods for Enhancing Penetration

Methods for enhancing penetration across the skin barrier first include hydration of the stratum corneum, which facilitates diffusion of hydrophilic molecules. Any form of occlusion, such as the allergen patches used by us^{51,65,66} and others,⁶⁷ hydrates the skin by accumulation of sweat.^{68,69} Also, a French group recently developed an alternative form of EPIT based on allergen delivery to the intact skin using an occlusive epidermal delivery system (Viaskin EDS).^{68,69,72} Initially developed for diagnostic purposes as an alternative system to the conventional Finn chamber used in atopy patch test,⁷³ Viaskin relies on the ability to deliver whole protein molecules to the skin.^{68,72} Perspiration, generated under an occlusive chamber, dissolves the lyophilized allergen protein loaded on the Viaskin EDS,^{68,72} and protein has been demonstrated to accumulate in the stratum corneum, where it efficiently targets immune cells of the

superficial skin layer⁷⁴ that rapidly migrate to the draining lymph nodes.⁶⁸ In murine studies, EPIT with Viaskin EDS has proven efficacy equivalent to SCIT in preventing allergic airway reactions on inhalative allergen challenge.⁶⁸ Skin penetration may also be enhanced by adding so-called penetration enhancers, such as salicylic acid,⁶⁷ or by packing the antigen into lipid-based colloidal systems.⁷⁵ Last but not least, skin penetration can be enhanced by microporation, either using a microneedle patch^{45,76} or a laser.^{77,78}

Although all of these methods enhance skin penetration of allergens, it remains to be seen to which degree each of these methods also activates keratinocytes, which importantly interact with LCs. It may be speculated that the different outcomes of these methods, such as the relative inefficacy of the Viaskin EDS chamber, may well be explained by the assumption that hydration alone does not activate keratinocytes as much as tape stripping or abrasion. In fact, a heavily disrupted skin barrier has been observed to polarize the immune response toward Th1, whereas slight skin barrier disruption rather induces a noninflammatory Th2/Treg dominated response.²⁴ The evidence in this area is conflicting. In mouse models of peanut allergy, intact skin and not stripped skin was found to be crucial for efficacy and safety of immunotherapy.⁷⁹ In our mouse models with ovalbumin, no therapeutic effect was observed if the skin was not tape stripped before allergen application.²³

We have recently use microneedle patches and laser microporation of the stratum corneum to enhance allergen penetration into the epidermis (Betschart E, Spina L, Tay F, et al: LASER microporation for epicutaneous allergen immunotherapy. Submitted for publication).²⁵ In these clinical trials, we found that once the allergen had passed across the stratum corneum and therefore penetrated into the live layers of the epidermis, the basal membrane represented no barrier to diffusion into the dermis. Even when merely the upper 10 μm of the epidermis were perforated, that is, only the stratum corneum, we could observe formation of hives only minutes after application of allergens (unpublished observations).

Adjuvants in Epicutaneous Immunotherapy

Adjuvants in EPIT represent another strategy to enhance efficacy. Aluminum salts, today still the adjuvant used in most marketed vaccines,⁸⁰ is not suitable for epicutaneous administration.⁸¹ Thus far, cholera toxin and heat-labile enterotoxin have been successfully used as adjuvants in epicutaneous vaccination against infectious diseases of mice and humans.^{43,82,83} However, imidazoquinolines (TLR7 or TLR8 ligands) and CpG (TLR9 ligand) are currently being tested as adjuvants for epicutaneous vaccination against cancer.^{49,84} Our group recently tested the immune-enhancing and immunomodulatory potential of diphenylcyclopropanone when used as adjuvant in EPIT.²³

Outlook for Allergen Epicutaneous Immunotherapy in Humans

With several placebo-controlled double-blind clinical trials confirming the efficacy of EPIT in allergy,^{26,61,65–67} there remains little doubt that this method works as a proof of principle. Our clinical trial program has also shown that the allergen dose is an important efficacy parameter,^{65,66} but the allergen dose used in skin patches cannot readily be increased beyond a certain concentration. We used 30 μg of major grass pollen allergen Phl p 1 per patch, corresponding to 1 mL of the 10-fold concentration that is used for the skin prick solution. A higher concentration would not only generate considerable material costs, but the application to any accidentally impaired skin barrier would represent a considerable risk for systemic allergic effects. Future research should therefore focus on enhancing penetration of the stratum corneum into the

viable layers of the skin where LCs reside, and also on adjuvants suitable for epicutaneous administration.

INTRADERMAL ALLERGEN IMMUNOTHERAPY

In light of the observation that allergen delivery to the epidermis also immediately delivers the allergens by diffusion further down to the dermis, we cannot disentangle whether symptom amelioration in our clinical trials on EPIT actually worked via epidermal delivery to LCs, via dermal delivery to dermal DCs, or by both. In fact, the dermis with its high density of APCs represents an interesting immunotherapy route. Intradermal allergen delivery was shown to reduce hay fever symptoms already in 1926 by Phillips.⁵⁵ A recent clinical trial showed that very low allergen doses delivered to the dermis could induce allergen tolerance.²⁶ Also, intradermal injection of peptides derived from Fel d 1 and other allergens have shown profound clinical symptom amelioration.¹² Also, it should not be forgotten that the higher perfusion rate of the dermis produces per gram of tissue markedly more lymph than is produced in the rather poorly perfused subcutis. This causes intradermally injected substances to be drained into lymph nodes significantly faster than when subcutaneously injected.^{85,86} The intradermal route is also gaining attention for other vaccines, such as influenza vaccines.^{87,88}

SUMMARY AND FUTURE DIRECTIONS

AIT needs further improvement because the current protocols SCIT and SLIT suffer from long treatment duration and allergic side effects, so that very few patients with allergy choose to undergo these therapies and treatment adherence is low. AIT can be improved by modification of the allergen, by improving the adjuvant, or by changing the route of administration. The ideal route of administration is characterized by a high density of potent APCs and a low density or ideally even the absence of mast cells responsible for local side effects and of vasculature responsible for systemic allergic side effects. The intralymphatic route is interesting in that lymph nodes contain the highest density of DCs found in the human body. We and others demonstrated that as few as three allergen injections into a lymph node are sufficient to ameliorate allergy symptoms.

Also, allergen application to the epidermis is interesting in that epithelia contain a high number of DCs, but neither mast cells nor vasculature. We found, however, that for allergens the basal membrane represents no diffusion barrier toward the dermis, so that epicutaneously applied allergen still reaches the dermis. However, when the epidermis was prepared by adhesive tape stripping, we found EPIT to be safe. Only six patch applications were sufficient to ameliorate allergy symptoms. However, if the epidermis was prepared by abrasion, we observed several systemic allergic reactions. For EPIT we conclude that skin preparation is of key importance. First, the stratum corneum is the main diffusion barrier and must be made permeable for allergens to reach the live epidermis where APCs reside. Second, physical or chemical trauma to the epidermis provides signals and an immunologic milieu for DCs that modulate the efficacy of AIT. For both novel routes of AIT extensive clinical trial programs are under way and are likely to provide for interesting new treatment options in allergy.

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