

Review Article

Immune system network in allergic diseases during sublingual immunotherapy: The role of oral mucosal tissue

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Abstract. Specific sublingual immunotherapy (SLIT) represents an approach currently available to redirect inappropriate immune response in atopic patients. Since oral mucosal tissue displays high affinity for allergens, it is conceivable that the sublingual administration route might induce immunological tolerance towards allergens involving cells and mediators specific of oral and intestinal mucosa. The presence in oral mucosa of dendritic cells (DCs) which express the high-affinity receptor for immunoglobulin (Ig)E (FcεRI) seems to suggest that the generation of T regulatory cells in periphery is orchestrated by a particular subset of DCs. It seems that repeated stimulation of naïve CD4 T cells with allogenic immature DCs induce Tr1 cell maturation. Nevertheless, other cells are involved in this process, such as Toll Like Receptors (TLR), Major Histocompatibility Complex I and II (MHC I and II) and costimulatory molecules, such as CD40, CD 80/B7.1 and CD 86/B7.2. An increase of serum IgG4 and IgA, a reduced number of inflammatory cells infiltrating target organs, as well as a reduction of eosinophilic cationic protein and a T cell suppression in the peripheral blood also occur with SLIT. All these molecules orchestrate the immune network within the regional immune system, recreating a favourable environment for the induction of tolerance operated by SLIT.

Keywords: Oral mucosa, dendritic cells, Sublingual immunotherapy (SLIT), Immunological tolerance

1. Introduction

Specific immunotherapy (SIT) has been shown to be safe and effective in the treatment of clinically significant respiratory IgE-mediated diseases [1,2] and it represents the only approach currently available to redirect inappropriate immune response in atopic patients [3]. The risk of severe systemic side effects and the need for frequent injections has limited the application of the subcutaneous immunotherapy (SCIT),

especially in children in which sublingual-swallow immunotherapy (SLIT) may represent an alternative.

In fact SLIT has been shown to be safe and effective in several double blind placebo-controlled studies in children affected by asthma and rhinitis [4–6] and in 2001 the guidelines on allergic rhinitis and its impact on asthma (ARIA) extended the indications of SLIT also to children [7].

1.1. Oral mucosa dendritic cells (DCs): What is known in allergy?

All dendritic cells (DCs) and subtypes display a few common features, such as their localization in the

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Table 1
Dendritic Cells (DCs) subtypes in skin and mucosal epithelium

DC subtypes	Mediators production	Localization
Skin DCs/ Myeloid DCs/ LCs	Proinflammatory soluble mediators, chemotactic factors	Basal layers of the upper epidermis
Inflammatory dendritic epidermal cells (IDECs)	Proinflammatory cytokines (TNF- α , IL-8), chemokines and soluble factors (IL-12)	Inflamed epidermis
Dermal DCs		Dermis
Mucosal DCs/ oral DCs	Anti-inflammatory cytokines (IL-10, TGF- β)	Oral mucosal epithelium
Nasal DCs	Proinflammatory (TNF- α , IL-6) and anti-inflammatory cytokines (IL-10)	Nasal mucosal epithelium
Plasmacytoid DCs	IFN IL-10	Epidermal skin (patients with psoriasis or allergic contact dermatitis, but nearly absent in patients with atopic dermatitis), nasal mucosa, peripheral blood

body sites neighboring the exterior, which is associated directly with their pivotal role as sentinels of the immune system (Table 1). Another, unique feature is their capability to prime naive T cells and direct the nature of T cell responses.

Human DCs can be divided into two major subtypes, myeloid DCs (MDCs) and plasmacytoid DCs (PDCs). They are interferon (IFN)-producing cells recognizing viral antigens by Toll-like receptor 7 and 9 (TLR-7 and TLR-9) [8]. One of the most impressive examples for these variations is DCs which express the high-affinity receptor for immunoglobulin (Ig)E (Fc ϵ RI). Depending upon the context (i.e., cell type and location of Fc ϵ RI-bearing DCs), allergic immune responses can be prevented, as it happens in the oral mucosa during sublingual immunotherapy, due to the existence of Fc ϵ RI-positive DCs [9], or promoted, as it occurs in the skin of patients with atopic dermatitis (AD) [10–12].

Although the oral mucosal epithelium is exposed to high numbers of bacterial products and allergens derived from food, chronic allergic inflammatory reactions are observed less frequently at this site [12]. This is in contrast to other mucosal surfaces such as the nasal and bronchial mucosa, where local chronic allergic and inflammatory reactions often occur [13,14].

1.2. Dendritic cells modification: What is known during sublingual immunotherapy?

In the last years, some studies indicated that the mechanism of SLIT might be based on the increase of number and activity of regulatory T cells. Regulatory T cells appear to control the development of autoimmune diseases and transplant rejection and may also play a critical role in controlling asthma and

allergy. In particular, type 1 regulatory T cells (Tr1) are a subset of CD4⁺ T cells that produce high levels of IL-10, low levels of IL-2 and no IL-4 [15]. It has been reported that they consistently represent the dominant subset specific for common environmental allergens in healthy individuals in contrast to the allergen-specific IL-4-secreting T cells frequently found in allergic individuals [16–18]. Specific immunotherapy, by modifying Tr1 compartment, would then restore the natural immune tolerance to antigens such as aeroallergens [19–21].

Accumulating evidence suggests that the generation of regulatory T cells in periphery is orchestrated by a particular subset of dendritic cells (DCs), whose phenotype and modality of function are topics of intense study. Early reports showed that repeated stimulation of naive CD4 T cells with allogenic immature DCs induce Tr1 cells [22,23]. Subsequently, it has been demonstrated that a specialized subset of DCs, termed tolerogenic DCs, can prime T cells to differentiate in Tr1 cells, thus promoting tolerance rather than immunity [24] and several protocols have been developed to generate Tr1 cells *in vitro* by using tolerogenic DCs [25].

To elucidate the immunological changes induced by SLIT, Angelini and colleagues [26] studied the function of DCs in asthmatic patients receiving SLIT for 12 months. They observed a statistically significant reduction over time of the expression of CD86, an important costimulatory molecule for T cells, on MDCs. Differently, CD80 expression, as well as HLA-DR expression, was substantially unchanged. CD86 appeared indeed to be more important than CD80 for the induction of the allergic immune response [27,28]. Therefore, the reduction of CD86 expression that Angelini and colleagues observed

during SLIT [26] might have an important role in the induction of tolerance or in the suppression of immune response to allergens.

Moreover, Angelini and colleagues observed a contemporary decrease of IL-12 production by DCs after 12 months [26].

It has been shown recently that IL-10 can induce the differentiation of T cells with antigen-specific regulatory activity, directly or via induction of an IL-10 producing subset of DCs [29–32]. Therefore, it is conceivable that an increase of IL-10 production by the DCs during the first months of SLIT might drive an expansion of the T regulatory cell population, which would be responsible for the clinical improvement usually observed after the first year of immunotherapy. This interpretation is supported by the recent work of Koya and colleagues [33], who demonstrated that DCs treated with IL-10 produced, in turns, considerable amounts of IL-10, and that the adoptive transfer of IL-10-treated DCs leads to an increase of CD4+IL-10+T cells in the lung of sensitized recipient mice.

1.3. SLIT and oral tolerance: An update and future target

Sublingual administration of vaccines to prevent chronic infectious diseases [34] and of allergens as in SLIT is independent from swallowing the allergen and from any contact of the allergen with the mucosa of the lower gastrointestinal tract [35,36].

Data about the exact mode of action of SLIT are limited but are most likely very similar to the mechanisms assumed to be responsible for the effect of its subcutaneous variant. It is believed that it is more than likely that IgE receptor-bearing DCs in the oral mucosa take up allergens from allergen solutions and tablets and induce T cell responses. In contrast to subcutaneous allergen specific immunotherapy, effects observable locally at mucosal sites predominate during SLIT. An increase of serum IgG4 and IgA, a reduced number of inflammatory cells infiltrating target organs, as well as a reduction of eosinophilic cationic protein and a very heterogeneous influence on T cells in the peripheral blood in terms of T cell suppression occur with SLIT [35,37,38]. Other groups reported an enhancement of Foxp3+CD4+CD25+ T regulatory cells (Treg) and IL-10 in the peripheral blood [39], as well as enhancement of IL-18 and the signaling lymphocytic activation molecule (SLAM) in

peripheral blood mononuclear cells (PBMCs) after SLIT, which suggests an up regulation of tolerance induction and a down regulation of Th2 immune responses [40].

Concerning clinical studies, a meta-analysis that covered ten studies conducted in a total of 577 children [41] revealed that SLIT represents an effective treatment alternative to the subcutaneous route in the clinical practice.

Various vector systems are used in immunotherapy to optimize the delivery of the antigen to target cells. In addition, substances capable of redirecting Th2 prone immune responses into modified, more Th1 dominated immune responses are used as adjuvants to provide cosignals to T cells [42]. Most of these tools target innate immunity receptors such as TLRs. In this context, miming the physiologic way of tolerance induction toward bacterial products in the oral mucosa, the addition of TLR ligands, such as monophosphoryl lipid A (MPL) or Cytosine-phosphate-Guanine sites (CpG), to the vaccine represents a very successful strategy [43–45]. Data from human oral DCs stimulated with MPL *in vitro* hold promise that the combination of allergens with TLR ligands might increase the efficacy of SLIT [43,45].

Recently Lombardi T et al. demonstrated in an *in vitro* study that the TLR2 agonist synthetic lipopeptide Pam3CSK4 is the most powerful inducer of IL-12p40 and IL-10 secretion in murine models. In addition, they demonstrated a similar effect of Pam3CSK4 on purified CD11b+ CD11c- MHC class II+ oral myeloid DCs, i.e. the main population of oral Antigen Presenting Cells (APCs) in mice [46]. Bacterial toxins are able to enter the cytosol and are therefore useful adjuvants to deliver antigens to major histocompatibility pathways [47]. However, the toxicity sometimes limits the application of bacterial toxins in the human system, so detoxified or nontoxic variants have been generated within the last years to circumvent this problem [47].

In the context of adjuvants applicable to mucosal surfaces, nonpathogenic food-grade bacteria, such as lactic acid bacteria [47], might represent a more physiologic alternative. Co-application of allergens with lactic acid bacteria to the mucosa in a murine model induced increased levels of allergen-specific IgG2 in the sera, as well as higher Interferon- γ production by Th1 cells. Sublingual application of 1,25-dihydroxyvitamin D3, dexamethason or *Lactobacillus plantarum* induced IL-10 production by DCs and

enhanced SLIT efficacy in mice [48,49].

Furthermore, approaches using virus like particles and plasmid DNA [45] represent other strategies that follow the physiologic ways of pathogen transmission. Plasmid-DNA lipid complexes applied intranasal have been shown to induce effective immunity against pathogens transferred by way of mucosal surfaces and hold promise of application to the oral mucosa in the future [50].

There are also some recent approaches aimed to optimize mucosal adhesion of antigens with the help of ovalbumin combined with maltodextrin to enhance mucosal adhesion. These studies revealed reduced airway hyperresponsiveness, IL-5 and IgE production in a mouse asthma model [51]. Also agents that increase the permeability of the oral mucosa, which differs depending on the region or grade of keratinization, have been developed to enhance antigen uptake [52].

In conclusion, the sublingual mucosa belongs to the sophisticated network of mucosal immune system, where tolerance induction towards a variety of antigens from commensal bacteria and nutritional products predominates to maintain local immunostasis. Oral mucosal tissue displays high permeability also for allergens, and orally applied allergen had been shown to retain within this process. Different cells are involved in this process, such as Langherans-like local dendritic cells, T Lymphocytes with suppressive function, TLR, MHC of I and II class and costimulatory molecules such as CD40, CD 80/B7.1 and CD 86/B7.2. All these molecules orchestrate the immunological network within the regional immune system, recreating an environment for the induction of tolerance operated by SLIT.

References

- [1] Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; 102 (4 Pt 1): 558-62.
- [2] La Rosa M, Lionetti E, Leonardi S, Salpietro A, Bianchi L, Salpietro C et al. Specific immunotherapy in children: the evidence. *Int J Immunopathol Pharmacol* 2011; 24(4 Suppl): 69-78.
- [3] Leonardi S, La Rosa M, Bellanti JA. Atopic disease, immune system and environment: which linkage? *Allergy Asthma Proc* 2007; 28: 410-17.
- [4] Bousquet J, Scheinmann P, Guinnee MT, Perrin-Fayolle M, Sauvaget J, Tonnel AB et al. Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to housedust mites: a double-blind, placebo-controlled study. *Allergy* 1999; 54(3): 249-60.
- [5] La Rosa M, Ranno C, Andr  C, Carat F, Tosca MA, Canonica GW. Double-blind placebo-controlled evaluation of sublingual swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999; 104 (2 Pt 1): 425-32.
- [6] Miraglia del Giudice M, Marseglia A, Leonardi S, La Rosa M, Salpietro C, Arrigo T et al. Allergic rhinitis and quality of life in children. *Int J Immunopathol Pharmacol* 2011; 24(4 Suppl): 25-8.
- [7] Bousquet J, Sch  nnemann HJ, Samolinski B, Demoly P, Bae-na-Cagnani CE et al. Allergic Rhinitis and its Impact on Asthma (ARIA): Achievements in 10 years and future needs. *J Allergy Clin Immunol* 2012; 130(5): 1049-62.
- [8] Banchereau J, Klechevsky E, Schmitt N, Morita R, Palucka K, Ueno H. Harnessing human dendritic cell subsets to design novel vaccines. *Ann NY Acad Sci* 2009; 1174: 24-32.
- [9] Novak N, Haberstock J, Bieber T, Allam JP. The immune privilege of the oral mucosa. *Trends Mol Med* 2008; 14: 191-8.
- [10] Leonardi S, Rotolo N, Vitaliti G, Spicuzza L, La Rosa M. IgE values and T lymphocyte subsets in children with atopic eczema/dermatitis syndrome (AEDS). *Allergy Asthma Proc* 2007; 28(5): 529-34.
- [11] Miraglia del Giudice M, Decimo F, Leonardi S, Maioello N, Amelio R, Capasso A et al. Immune dysregulation in atopic dermatitis. *Allergy Asthma Proc* 2006; 27(6): 451-5.
- [12] Novak N. New insights into the mechanism and management of allergic diseases: atopic dermatitis. *Allergy* 2009; 64(2): 265-75.
- [13] Miraglia del Giudice M, Marseglia GL, Leonardi S, Tosca MA, Marseglia A, Perrone L et al. Fractional exhaled nitric oxide measurements in rhinitis and asthma in children. *Int J Immunopathol Pharmacol* 2011; 24(4 Suppl): 29-32.
- [14] Leonardi S, Vitaliti G, Marseglia GL, Caimmi D, Lionetti E, Miraglia del Giudice M et al. Function of the Airway Epithelium in Asthma. *J Biol Regul Homeost Agents* 2012; 26(1): 41-8.
- [15] Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. *J Allergy Clin Immunol* 2009; 123(4): 735-46.
- [16] Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199(11): 1567-75.
- [17] Ozdemir C, Akdis M, Akdis CA. T regulatory cells and their counterparts: masters of immune regulation. *Clin Exp Allergy* 2009; 39(5): 626-39.
- [18] Rigoli L, Briuglia S, Caimmi S, Ferrau' V, Gallizzi R, Leonardi S et al. Gene-environment interactions in childhood asthma. *Int J Immunopathol Pharmacol* 2011; 24(4 Suppl): 41-7.
- [19] Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+ CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; 111(6): 1255-61.
- [20] Gardner LM, Thien FC, Douglass JA, Rolland JM, O'Hehir RE. Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 2004; 34(8): 1209-19.
- [21] Jutel M, Akdis CA. T-cell regulatory mechanisms in specific immunotherapy. *Chem Immunol Allergy* 2008; 94: 158-77.
- [22] Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 2000;

- 192(9): 1213-22.
- [23] Nguyen KD, Vanichsam C, Nadeau KC. Impaired IL-10-dependent induction of tolerogenic dendritic cells by CD4+CD25hiCD127lo/- natural regulatory T cells in human allergic asthma. *Am J Respir Crit Care Med* 2009; 180(9): 823-33.
 - [24] Rutella S, Danese S, Leone G. Tolerogenic dendritic cells: cytokine modulation comes of age. *Blood* 2006; 108(5): 1435-40.
 - [25] Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 2006; 212: 28-50.
 - [26] Angelini F, Pacciani V, Corrente S, Silenzi R, Di Pede A, Polito A et al. Dendritic cells modification during sublingual immunotherapy in children with allergic symptoms to house dust mites. *World J Pediatr* 2011; 7(1): 24-30.
 - [27] Tsuyuki S, Tsuyuki J, Einsle K, Kopf M, Coyle AJ. Costimulation through B7-2 (CD86) is required for the induction of a lung mucosal T helper cell 2 (TH2) immune response and altered airway responsiveness. *J Exp Med* 1997; 185(9): 1671-9.
 - [28] Larch  M, Till SJ, Haselden BM, North J, Barkans J, Corrigan CJ et al. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J Immunol* 1998; 161(11): 6375-82.
 - [29] Ciprandi G, Cirillo I, Fenoglio D, Marseglia G, Tosca MA. Sublingual immunotherapy induces spirometric improvement associated with IL-10 production: preliminary reports. *Int Immunopharmacol* 2006; 6(8): 1370-3.
 - [30] Steinbrink K, Graulich E, Kubsch S, Knop J, Enk AH. CD4(+) and CD8(+) anergic T cells induced by interleukin 10-treated human dendritic cells display antigen-specific suppressor activity. *Blood* 2002; 99(7): 2468-76.
 - [31] Sato K, Yamashita N, Baba M, Matsuyama T. Modified myeloid dendritic cells act as regulatory dendritic cells to induce anergic and regulatory T cells. *Blood* 2003; 101(9): 3581-9.
 - [32] Wakkach A, Fournier N, Brun V, Breittmayer JP, Cottrez F, Groux H. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity* 2003; 18(5): 605-17.
 - [33] Koya T, Matsuda H, Takeda K, Matsubara S, Miyahara N, Balhorn A et al. IL-10-treated dendritic cells decrease airway hyperresponsiveness and airway inflammation in mice. *J Allergy Clin Immunol* 2007; 119(5): 1241-50.
 - [34] Cuburu N, Kweon MN, Song JH, Hervouet C, Luci C, Sun JB et al. Sublingual immunization induces broad-based systemic and mucosal immune responses in mice. *Vaccine* 2007; 25(51): 8598-610.
 - [35] Holt PG, Vines J, Britten D. Sublingual allergen administration. I. Selective suppression of IgE production in rats by high allergen doses. *Clin Allergy* 1988; 18(3): 229-34.
 - [36] Miraglia del Giudice M, Leonardi S, Maiello N, Brunese FP. Food allergy and probiotics in childhood. *J Clin Gastroenterol* 2010; 44 (Suppl 1): 22-5.
 - [37] Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; 6(10): 761-71.
 - [38] Akdis CA, Barlan IB, Bahceciler N, Akdis M. Immunological mechanisms of sublingual immunotherapy. *Allergy* 2006; 61 (Suppl 81):11-4.
 - [39] Girtsman T, Jaffar Z, Ferrini M, Shaw P, Roberts K. Natural Foxp3(+) regulatory T cells inhibit Th2 polarization but are biased toward suppression of Th17-driven lung inflammation. *J Leukoc Biol* 2010; 88 (3): 537-46.
 - [40] Savolainen J, Nieminen K, Laaksonen K, Laiho T, Jacobsen L, Laheesmaa R et al. Allergen-induced in vitro expression of IL-18, SLAMF6 and GATA-3 mRNA in PBMC during sublingual immunotherapy. *Allergy* 2007; 62(8): 949-53.
 - [41] Penagos M, Compalati E, Tarantini F, Baena-Cagnani R, Huerta J, Passalacqua G et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol* 2006; 97(2): 141-8.
 - [42] de Brito CA, Goldoni AL, Sato MN. Immune adjuvants in early life: targeting the innate immune system to overcome impaired adaptive response. *Immunotherapy* 2009; 1(5): 883-95.
 - [43] Allam JP, Stojanovski G, Friedrichs N, Peng W, Bieber T, Wenzel J et al. Distribution of Langerhans cells and mast cells within the human oral mucosa: new application sites of allergens in sublingual immunotherapy? *Allergy* 2008; 63(6): 720-7.
 - [44] Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T et al. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J Allergy Clin Immunol* 2008; 121(2): 368-74.
 - [45] Huang CF, Wang CC, Wu TC, Chu CH, Peng HJ. Effect of sublingual administration with a native or denatured protein allergen and adjuvant CpG oligodeoxynucleotides or cholera toxin on systemic T(H)2 immune responses and mucosal immunity in mice. *Ann Allergy Asthma Immunol* 2007; 99(5): 443-52.
 - [46] Lombardi V, Van Overtvelt L, Horiot S, Moussu H, Chabre H, Louise A et al. Toll like receptor 2 agonist Pam3csk4 enhances the induction of antigen-specific tolerance via the sublingual route. *Clin Exp Allergy* 2008; 38(11): 1819-29.
 - [47] Mascarell L, Van Overtvelt L, Moingeon P. Novel ways for immune intervention in immunotherapy: mucosal allergy vaccines. *Immunol Allergy Clin North Am* 2006; 26(2): 283-306.
 - [48] Van Overtvelt L, Lombardi V, Razafindratsita A, Saint-Lu N, Horiot S, Moussu H et al. IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. *Int Arch Allergy Immunol* 2007; 145(2): 152-62.
 - [49] Van Overtvelt L, Moussu H, Horiot S, Samson S, Lombardi V, Mascarell L et al. Lactic acid bacteria as adjuvants for sublingual allergy vaccines. *Vaccine* 2010; 28(17): 2986-92.
 - [50] Klavinskis LS, Barnfield C, Gao L, Parker S. Intranasal immunization with plasmid DNA-lipid complexes elicits mucosal immunity in the female genital and rectal tracts. *J Immunol* 1999; 162(1): 254-62.
 - [51] Razafindratsita A, Saint-Lu N, Mascarell L, Berjont N, Bardon T, Betbeder D et al. Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. *J Allergy Clin Immunol* 2007; 120(2): 278-85.
 - [52] Kurosaki Y and Kimura T. Regional variation in oral mucosal drug permeability. *Crit Rev Ther Drug Carrier Syst* 2000; 17(5): 467-508.