

MUCOSAL IMMUNITY

Alessandra Pernis

P&S 9-435
X53763
abp1@columbia.edu

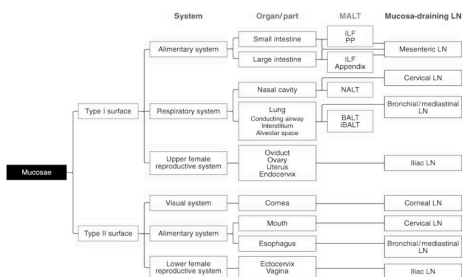
- CHALLENGES FACED BY THE MUCOSAL SYSTEM
- SPECIALIZATION OF CELLS INVOLVED IN MUCOSAL IMMUNITY
- ORGANIZATION OF THE MUCOSAL IMMUNE SYSTEM
- CLINICAL IMPLICATIONS

DEFINITIONS

MALT= MUCOSA-ASSOCIATED LYMPHOID TISSUE

MALT is the highly specialized immune system which protects mucosal surfaces. The lymphoid elements associated with different mucosal sites share organizational as well as functional similarities. It is the largest mammalian lymphoid organ system and in an adult it comprises approximately 80% of all lymphocytes.

SUBDIVISIONS OF THE MUCOSA



Iwasaki A. 2007.
Annu. Rev. Immunol. 25:381-418

ILF=Isolated lymphoid follicle
PP=Peyer's Patches

Distinct features of type I and type II mucosal surfaces

	Type I	Type II
Epithelia	Simple	Stratified
Presence of MALT	+	-
Presence of pIgR	+	-
Major Ig isotype	IgA	IgG
Goblet cells	+	-

pIgR=polymeric Ig receptor

THE CHALLENGES

- MOST FREQUENT PORTAL OF ENTRY FOR HARMFUL SUBSTANCES. THUS THE MALT HAS TO MOUNT AN EFFECTIVE RESPONSE AGAINST A VAST NUMBER OF POTENTIAL PATHOGENS.
- THE MUCOSAL MEMBRANES OF THE DIGESTIVE TRACT MUST ALLOW FOR THE ABSORPTION OF NUTRIENTS BY THE HOST. THUS THE MALT MUST REMAIN HYPORESPONSIVE TO AN ENTIRE ARRAY OF HARMLESS SUBSTANCES.

SPECIALIZED COMPONENTS OF MALT

B CELLS

- HUMORAL RESPONSES ARE CENTRAL TO AN EFFECTIVE MUCOSAL IMMUNITY.
- THE MAIN HUMORAL MEDIATORS OF SPECIFIC MUCOSAL IMMUNITY ARE SECRETORY IgA AND, TO A LESSER EXTENT, SECRETORY IgM.
- THE NORMAL INTESTINAL MUCOSA CONTAINS AT LEAST 20 TIMES MORE IgA+ THAN IgG+ LYMPHOCYTES.

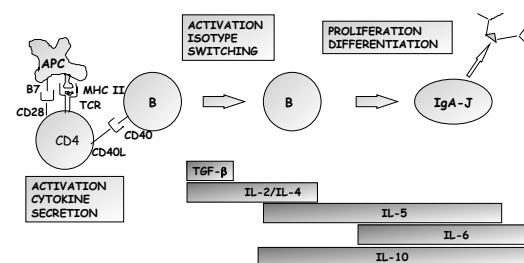
CRITICAL FEATURES OF SECRETORY IgA

- RESISTANCE AGAINST COMMON INTESTINAL PROTEASES
- INABILITY TO INTERACT WITH COMPLEMENT OR CELLS IN A WAY TO CAUSE INFLAMMATION

MECHANISMS OF PROTECTION BY SIgA AT MUCOSAL SURFACES

- INHIBITION OF ADHERENCE
- VIRUS NEUTRALIZATION
- NEUTRALIZATION OF ENZYMES AND TOXINS
- IMMUNE EXCLUSION AND INHIBITION OF ANTIGEN ABSORPTION

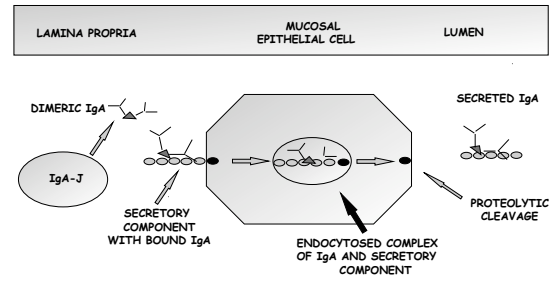
FACTORS CONTROLLING IgA ISOTYPE SWITCHING



FACTORS CONTROLLING THE SECRETION OF IgA: THE J CHAIN

- THE J CHAIN IS A 15 KD POLYPEPTIDE THAT IS DISULFIDE-BONDED TO THE TAIL-PIECES OF BOTH IgM AND IgA
- IgA SECRETING B CELLS IN THE BONE MARROW DO NOT EXPRESS THE J CHAIN AND THUS SECRETE IgA MONOMERS
- THE MAJORITY OF IgA PRODUCING B CELLS IN THE MUCOSA EXPRESS THE J CHAIN AND THUS PRODUCE DIMERIC IgA
- THE J CHAIN STABILIZES THE MULTIMERS AND IT APPEARS TO DETERMINE THE POLYMERIC IgA AND IgM STRUCTURE WHICH ALLOWS POLYMERIC Igs TO COMPLEX WITH THE SECRETORY COMPONENT

FACTORS CONTROLLING THE SECRETION OF IgA: THE SECRETORY PIECE (POLYMERIC Ig RECEPTOR)

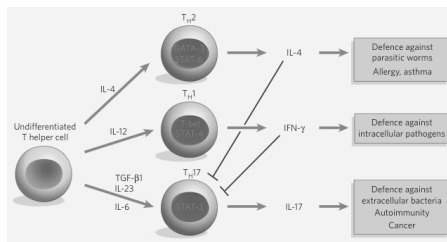


T CELLS

LAMINA PROPRIA LYMPHOCYTES

- LYMPHOCYTES WHICH ARE SCATTERED DIFFUSELY THROUGHOUT THE LAMINA PROPRIA OF THE INTESTINE. (LAMINA PROPRIA=LAYER OF CONNECTIVE TISSUE BETWEEN THE EPITHELIUM AND THE MUSCULARIS MUCOSA)
- LARGEST SINGLE T-CELL SITE IN HUMANS. MOST OF THE T CELLS WITHIN THE LAMINA PROPRIA ARE CD4+.

TH1, TH2 or TH17?



From: Tato and O'Shea Nature 441:166 (2006)

INTRAEPITHELIAL LYMPHOCYTES (IELs)

- IELs ARE LYMPHOCYTES WHICH ARE INTERSPERSED BETWEEN THE COLUMNAR EPITHELIAL CELLS OF THE VILLI IN THE SMALL AND LARGE INTESTINE
- IN HUMANS, MOST OF THE IELs ARE CD8+ T CELLS. APPROXIMATELY 10% OF IELs ARE γδ CELLS
- BOTH THE γδ AND THE αβ TCR+ IELs SHOW LIMITED DIVERSITY OF T CELL RECEPTOR

FUNCTIONAL PROPERTIES OF IELs

- **FIRST IMMUNE CELL LINE OF DEFENSE IN THE INTESTINE**
- **DISPLAY CYTOTOXIC ACTIVITY**
- **SECRETE LARGE AMOUNTS OF CYTOKINES ESPECIALLY IFN- γ AND TNF- α**
- **MODULATE THE KINETICS OF EPITHELIAL CELL RENEWAL**

REGULATORY T CELLS

- **TH3 CELLS: A POPULATION OF CD4+T CELLS THAT PRODUCE TGF- β . ISOLATED FROM MICE FED LOW DOSE OF ANTIGEN FOR TOLERANCE INDUCTION**
- **TR1 CELLS: A POPULATION OF CD4+T CELLS THAT PRODUCE IL-10. CAN PRODUCE SUPPRESSION OF EXPERIMENTAL COLITIS IN MICE**
- **CD4+CD25+ REGULATORY T CELLS: A POPULATION OF CD4+T CELLS THAT CAN PREVENT AUTOREACTIVITY IN VIVO.**

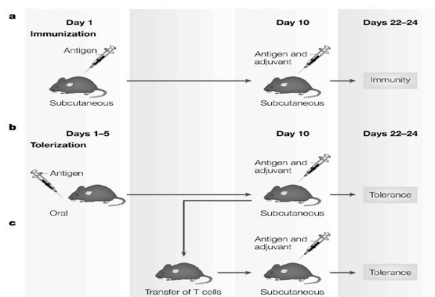
REGULATORY T CELLS

- **CD8+SUPPRESSOR T CELLS: THE FIRST IDENTIFIED POPULATION OF REGULATORY T CELLS THOUGHT TO BE INVOLVED IN ORAL TOLERANCE.**
- **$\gamma\delta$ T CELLS: STUDIES IN MICE INDICATE THAT THEY HAVE AN IMPORTANT ROLE IN SOME MODELS OF ORAL TOLERANCE.**

ORAL TOLERANCE

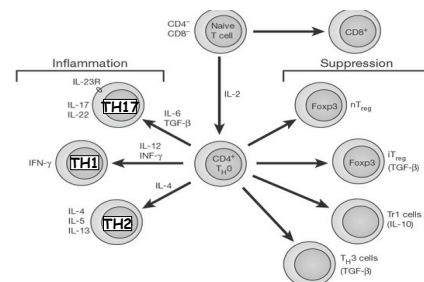
- **ORAL ADMINISTRATION OF A PROTEIN ANTIGEN MAY LEAD TO SUPPRESSION OF SYSTEMIC HUMORAL AND CELL-MEDIATED IMMUNE RESPONSES TO IMMUNIZATION WITH THE SAME ANTIGEN.**
- **POSSIBLE MECHANISMS:**
 - INDUCTION OF ANERGY OF ANTIGEN-SPECIFIC T CELLS
 - CLONAL DELETION OF ANTIGEN-SPECIFIC T CELLS
 - SELECTIVE EXPANSION OF CELLS PRODUCING IMMUNOSUPPRESSIVE CYTOKINES (IL-4, IL-10, TGF- β)

ORAL TOLERANCE



Nature Reviews | Immunology

The relative balance between effector T cells and regulatory T cells determines intestinal immunity vs tolerance



R. J. Xavier & D. K. Podolsky *Nature* 448, 427-434 (26 July 2007)

EPITHELIAL CELLS

M CELLS



Scanning electron microscopy of a single microdissected dome (a) of a murine Peyer's patch. The M cells are identified by their relatively short, dark brush border; they are restricted to the dome epithelium (upper half in b). Crypts (arrows) are opening to the cleft between the dome and the neighboring villi.

From: Gebert et al., *Am. J. Pathol.* 154:1573, 1999

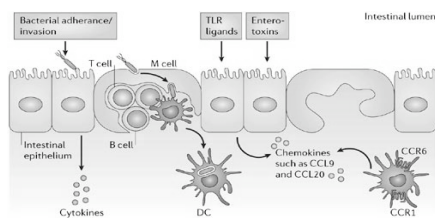
CHARACTERISTICS OF M CELLS

- M ("membrane-like") CELLS ARE SPECIALIZED EPITHELIAL CELLS WHICH OVERLIE LYMPHOID FOLLICLES DOMES ALONG THE LENGTH OF THE SMALL AND LARGE INTESTINE.
- STRUCTURAL FEATURES INCLUDE:
 - FEW SHORT IRREGULAR MICROVILLI
 - ABUNDANT ENDOCYTIC VESICLES
 - LOW LYSOSOMAL CONTENT
 - DISTINCTIVE GLYCOCALIX
 - BINDING SITES FOR SECRETORY IgA BUT NO SC
 - POCKETS IN THE BASOLATERAL SURFACE

FUNCTIONS OF M CELLS

- ANTIGEN SAMPLING
- PORTAL OF ENTRY FOR SELECTED PATHOGENS

FUNCTIONS OF FAE ENTEROCYTES



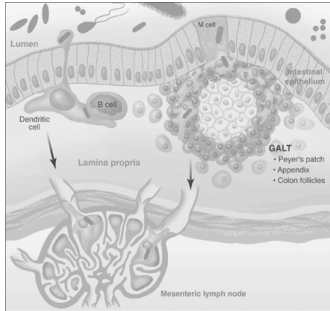
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Neutra et al. *Nature Reviews Immunology* 6, 148–158 (February 2006) | doi:10.1038/nri1777

nature
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DENDRITIC CELLS

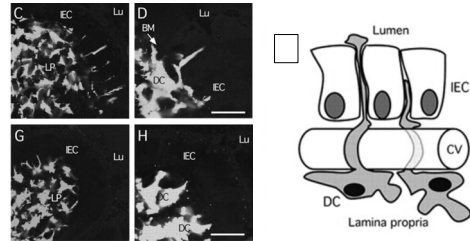
Dendritic cell-mediated transport of commensal bacteria in the gut



Dendritic cells can sample Antigen indirectly via M-cell transcytosis (right) or directly via processes that extend across the epithelial barrier (left). DCs present antigen to B- and T-cells, either directly within the lamina propria or following trafficking to the regional lymph nodes

From: Kraehenbuhl and Corbett, *Science* 303:1624, 2004

Role of CX₃CR1 in Luminal Sampling by Gut DCs



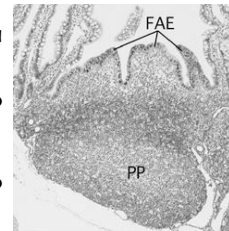
CX₃CR1 is a chemokine receptor whose ligand is an unusual chemokine; rather than secreted, it is membrane-bound

From: Niess et al., *Science* 307:254, 2005

ORGANIZATION OF MALT

INDUCTIVE SITES=PEYER'S PATCHES

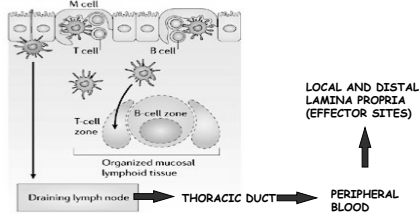
- ORGANIZED MUCOSAL LYMPHOID FOLLICLES WHICH LACK AFFERENT LYMPHATICS
- PEYER'S PATCHES ARE FOUND IN THE SMALL INTESTINE
- FOLLICLES SIMILAR TO PEYER'S PATCHES ARE FOUND IN THE APPENDIX, IN THE REST OF THE GI TRACT AND IN THE RESPIRATORY TRACT



From: Iwatsukit et al., *Histochem. Cell Biol.* 117:1363, 2001

CELLULAR TRAFFIC IN ORGANIZED MUCOSAL LYMPHOID TISSUES

Follicle-associated epithelium: small intestine, colon, rectum, tonsils and adenoids

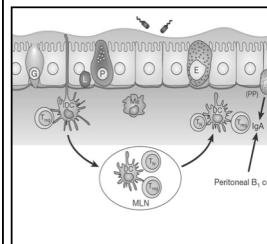


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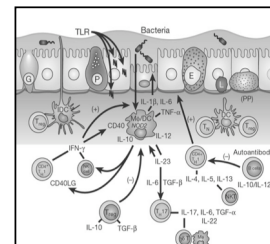
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Response to harmless pathogens



E=enterocyte
G=goblet cell
P=paneth cell

Response to harmful pathogens



R. J. Xavier & D. K. Podolsky *Nature* 448, 427–434 (26 July 2007)

CLINICAL IMPLICATIONS

IgA DEFICIENCY

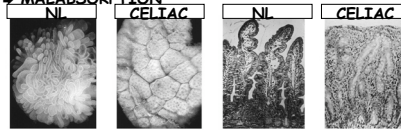
- IT IS THE MOST COMMON PRIMARY IMMUNODEFICIENCY
- IT IS USUALLY DEFINED BY A SERUM IgA CONCENTRATION OF LESS THAN 50 µg/ml
- IgA DEFICIENT INDIVIDUALS OFTEN APPEAR PERFECTLY HEALTHY AND ARE IDENTIFIED
 - UPON SERVING AS BLOOD DONORS
 - UPON UNDERGOING ANAPHYLACTIC SHOCK WHEN RECEIVING BLOOD TRANSFUSIONS

CLINICAL MANIFESTATIONS OF IgA DEFICIENCY

- INCREASED INCIDENCE OF INFECTIONS
 - UPPER AND LOWER RESPIRATORY TRACT
 - GASTROINTESTINAL
- HIGHER INCIDENCE OF AUTOIMMUNE DISEASES
- HIGHER INCIDENCE OF ALLERGIC DISEASES
- HIGHER INCIDENCE OF CELIAC DISEASE

CELIAC DISEASE

- CELIAC DISEASE IS A T CELL MEDIATED IMMUNE DISEASE OF THE SMALL INTESTINE TRIGGERED BY GLUTEN
- MAJOR FEATURES:
 - ➔ VILLOUS ATROPHY WITH A LYMPHOCYTIC INFILTRATE
 - ➔ INCREASED EPITHELIAL PROLIFERATION WITH CRYPT HYPERPLASIA
 - ➔ MALABSORPTION

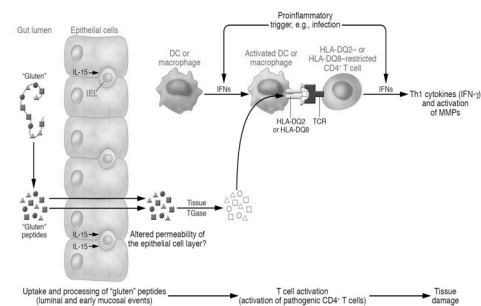


Kagnoff, M. F. J. Clin. Invest. 2007;117:41-49

CELIAC DISEASE: IMMUNOLOGIC FEATURES

- ANTIGEN: GLUTEN (gliadin and glutenins)
- IT IS ASSOCIATED WITH HLA-DQ2 OR HLA-DQ8 RESTRICTED LAMINA PROPRIA CD4+ T CELLS THAT RECOGNIZE GLUTEN AND SECRETE INTERFERON γ (98% OF PEOPLE WILL CARRY THESE HAPLOTYPES)
- GLIADIN IS A SUBSTRATE OF TISSUE TRANSGLUTAMINASE (TRANSFORMS POSITIVELY CHARGED GLUTAMINES TO NEGATIVELY CHARGED GLUTAMIC ACID)
- INCREASED B CELL ACTIVITY
 - ANTIBODIES AGAINST GLIADIN (IgA-A6A, IgG-A6A)
 - ENDOMYSIAL ANTIBODY (IgA-EMA)
 - TISSUE TRANSGLUTAMINASE (IgA-tTG)

CELIAC DISEASE PATHOGENESIS

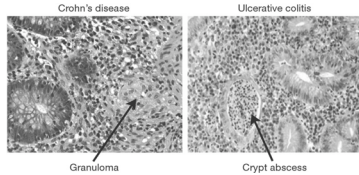


Kagnoff, M. F. J. Clin. Invest. 2007;117:41-49

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INFLAMMATORY BOWEL DISEASE (IBD)

- IBD IS A CHRONIC, RELAPSING AND REMITTING INFLAMMATORY CONDITION
- TWO OVERLAPPING PHENOTYPES:
 - ➔ CROHN'S DISEASE (CD), WHICH AFFECTS THE DISTAL SMALL INTESTINE AS WELL AS THE COLON IN A TRANSMURAL MANNER
 - ➔ ULCERATIVE COLITIS (UC), WHICH PREDOMINANTLY AFFECTS THE COLON IN A SUPERFICIAL MANNER

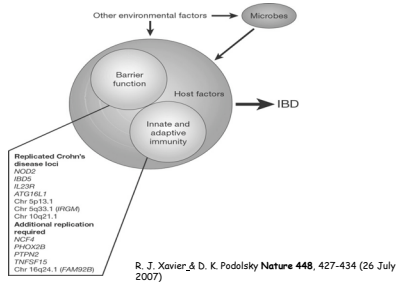


IBD: IMMUNOLOGIC FEATURES

- CELL-MEDIATED IMMUNITY (ACTIVE CD):
 - ➔ INCREASED NUMBER OF ACTIVATED MUCOSAL T CELLS SECRETING IFN- γ (TH1)
 - ➔ INCREASED MUCOSAL PRODUCTION OF CYTOKINES THAT ACTIVATE TH1 CELLS (IL-12 AND IL-18)
 - ➔ DEFECTS IN REGULATORY (IL-10 PRODUCING) T CELLS
- HUMORAL IMMUNITY: MASSIVE INCREASE IN THE NUMBER OF PLASMA CELLS AND IN IGG PRODUCTION (Igg2 IN CD AND Igg1 IN UC)
- IMBALANCE OF PRO-INFLAMMATORY (TNF- α , IL-1, IL-8, IL-12) AND ANTI-INFLAMMATORY CYTOKINES (IL-10, IL-4, IL-13)

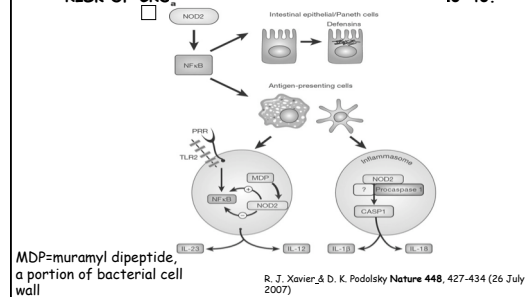
IBD PATHOGENESIS

THE ETIOLOGY IS UNKNOWN: ? DUE TO A DYSREGULATED MUCOSAL IMMUNE RESPONSE TO UNKNOWN ANTIGENS PRESENT IN COMMENSAL FLORA



NOD2 AND IBD PATHOGENESIS

MUTATIONS IN NOD2 (A CYTOSOLIC RECEPTOR FOR PATHOGENIC BACTERIAL SIGNALS) INCREASE THE RISK OF CROHN'S DISEASE BY A FACTOR OF 20-40.



IBD: EMERGING BIOLOGIC THERAPIES

- INHIBITORS OF PROINFLAMMATORY CYTOKINES
 - Anti-TNF therapies: infliximab
- ANTIINFLAMMATORY CYTOKINES
 - IL-10
 - IL-11
- ANTI-LEUKOCYTE ADHESION THERAPIES
 - Anti- α 4 integrin: Natalizumab ?
- INHIBITORS OF TH1/TH17 POLARIZATION
 - Anti-IL-12/IL-23
 - Anti-IL-18
 - Anti-IFN- γ

MUCOSAL IMMUNIZATION

MUCOSAL VACCINES

- VACCINES AGAINST MUCOSAL INFECTIONS MUST STIMULATE THE MALT IN ORDER TO BE EFFICACIOUS
- BECAUSE OF SUBCOMPARTMENTALIZATION WITHIN THE MALT, VACCINES MUST BE ADMINISTERED BY THE APPROPRIATE ROUTE
- NONREPLICATING ANTIGENS ARE OFTEN RELATIVELY INEFFICIENT IN YIELDING STRONG AND LONG-LASTING MUCOSAL ANTIBODY RESPONSES

MUCOSAL VACCINES

- NEW STRATEGIES FOR ANTIGEN DELIVERY:
 - ➔ LIVE ATTENUATED RECOMBINANT BACTERIA AND VIRUSES WITH KNOWN MUCOSAL TROPISM
 - ➔ PROTECTIVE VEHICLES, E.G. LIPOSOMES AND BIODEGRADABLE MICROSPHERES
 - ➔ MUCOSAL LECTIN-LIKE MOLECULES ENDOWED WITH IMMUNOSTIMULATORY PROPERTIES, E.G. CHOLERA TOXIN

EFFECT OF IMMUNIZATION ROUTE ON LOCAL AND DISTAL ANTIBODY RESPONSE

Immunogen	Route	Specific serum IgG	Responses of specific IgA antibodies*					References
			Small intestine	Large intestine	Cervix/vagina	Salivary glands	Nasal cavity	
Cholera toxin B subunit	Nasal	++++	ND	++	+++	-	+++	16,17, 114-116
	Oral	+++	++++	++	+++	+/-	+	
	Rectal	+++	ND	+++	+	+/-	ND	
Live attenuated <i>Salmonella typhi</i> Ty21a	Oral	++	+++	-	+/-	+/-	-	117,118
	Rectal	+	+/-	++	-	-	+/-	
Poliovirus vaccine	Oral	++++	+++	++	-	ND	+	119,120
	Colonic	++++	ND	+++	ND	ND	-	
	Vaginal	-	ND	ND	+++	ND	-	

*Responses are based on geometric mean post-vaccination increases in specific antibody corresponding to: +, >50-fold; ++, 25-49.9-fold; +++, 10-24-fold; +++, 5-9.9-fold; +, 2.5-4.9-fold; +/-, >2.5-fold in a minority of vaccine recipients; -, <2.5-fold in all vaccine recipients; ND, not determined.

Neutra et al. *Nature Reviews Immunology* 6, 148-158 (February 2006) | doi:10.1038/nri1777

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MUCOSAL IMMUNOTHERAPY

- STRATEGY TO ATTEMPT TO TREAT ILLNESSES RESULTING FROM IMMUNE REACTIONS AGAINST AUTOANTIGENS ENCOUNTERED IN NONMUCOSAL TISSUES
- HUMAN TRIALS HAVE BEEN CONDUCTED IN MULTIPLE SCLEROSIS, RHEUMATOID ARTHRITIS, UVEORETINITIS, AND TYPE I DIABETES

MUCOSAL IMMUNOTHERAPY

- POTENTIAL PROBLEMS:
 - ➔ LIMITED SUCCESS IN SUPPRESSING THE EXPRESSION OF AN ALREADY ESTABLISHED IMMUNE RESPONSE
 - ➔ MASSIVE AMOUNTS OF TOLEROGENS ARE REQUIRED
 - ➔ IMMUNOSUPPRESSIVE EFFECT IS OF SHORT DURATION

Table 3 | Oral tolerance in human diseases

Disease	Oral antigen	Dose	Prophylactic or therapeutic	Outcome	References
Food allergy	Allergen	Increasing dose over time	Therapeutic	About 50% of patients are successfully desensitized	130
Autoimmune uveitis	Sequestered retinal antigens, HLA-B27/DP	4 mg capsules 3 times a week for 12 weeks	Therapeutic	Marginal clinical benefit. All patients relapsed after cessation of treatment	131,132
	Retinal S-antigen, soluble retinal antigens	30 mg S-antigen or 50 mg soluble retinal antigens or both. Decreasing dose, starting from 3 times a week for 9 weeks, ending with once a week	Therapeutic	No benefit, with possible exacerbation of disease in patients receiving a mixture of soluble retinal antigens	133
Rheumatoid arthritis	Collagen	0.1 mg bovine type II collagen daily for 1 month, followed by 0.5 mg daily for 6 months	Therapeutic	No benefit	135
		20, 100, 500 or 2,500 µg chicken type I collagen daily for 24 weeks	Therapeutic	Clinically significant response at 20 µg dose	136
		0.05, 0.5 or 5 mg bovine type II collagen daily for 6 months	Therapeutic	Response at 0.5 mg	137
		0.5 mg bovine type II collagen daily for 2 months	Therapeutic	Response at 0.5 mg	138
		0.1 mg chicken type II collagen daily for 1 month, followed by 0.5 mg for 2 months	Therapeutic	Improvement in most clinical measures. 4 out of 28 patients had complete remission	139
Type 1 diabetes	Insulin	7.5 mg insulin	Prophylactic	No benefit	-
		2.5 mg or 7.5 mg insulin	Therapeutic	No benefit	140
Multiple sclerosis	Myelin	300 mg bovine myelin	Therapeutic	No clinically significant benefit	52,141

For trial results see National Institutes of Health News website. For further information, in contrast to experimental animal models, most human clinical trials have attempted to induce oral tolerance after the onset of disease (therapeutic). Treatments are prophylactic (the regimen of oral feeding began prior to the onset of clinical disease), whereas they are therapeutic if oral tolerance is initiated after the onset of disease. HLA-B27/DP, HLA-B27/miniDP.