Overview

1. "Basic" mechanisms of self-tolerance

- central tolerance
- peripheral tolerance

2. Mucosal immunology

- role of innate immunity in tolerance to foreign
- oral tolerance

3. Analyzing tolerance in clinical disease settings

- autoimmune disease vs. DTH reactions
- pathogenetic role of genetic and microbial factors
- where does tolerance fail and how can it be assessed?

Tolerance in clinical disease settings

Autoimmune disease versus hypersensitivity reactions

Autoimmune disease:
"A disease caused by a break-down in self-tolerance such that the adaptive immune system responds to self-antigens and mediates tissue damage" (A.K. Abbas)

Hypersensitivity:
"Excessive immune reactivity to a generelly innocuous (self- or foreign) antigen" (Tak W. Mak)

Hypersensitivity reactions may well be operative during autoimmune disease but collateral damage to self mediated through excessive immune reactivity to foreign is not an autoimmune disease !

When considering immunological tolerance in its completeness (i.e. not just as tolerance to self but also to foreign - where required), hypersensitivity reactions may also be caused or driven by failure in tolerance mechanisms !

Autoimmune diseases: Examples of AID caused by auto-antibodies

Disease	Target antigen	Mechanisms of disease	Clinicopathologic manifestations
Autoimmune hemolytic anemia	Erythrocyte membrane proteins (Rh blood group antigens, I antigen)	Opsonization and phagocytosis of erythrocytes	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins (gpIIb:IIIa integrin)	Opsonization and phagocytosis of platelets	Bleeding
Pemphigus vulgaris	Proteins in intercellular junctions of epidermal cells (epidermal cadherin)	Antibody-mediated activation of proteases, disruption of intercellular adhesions	Skin vesicles (bullae)
Vasculitis caused by ANCA	Neutrophil granule proteins, presumably released from activated neutrophils	Neutrophil degranulation and inflammation	Vasculitis
Goodpasture's syndrome	Noncollagenous protein in basement membranes of kidney glomeruli and lung alveoli	Complement- and Fc receptor–mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen	Inflammation, macrophage activation	Myocarditis, arthritis
Myasthenia gravis	Acetylcholine receptor	Antibody inhibits acetylcholine binding, down-modulates receptors	Muscle weakness, paralysis
Graves' disease (hyperthyroidism)	TSH receptor	Antibody-mediated stimulation of TSH receptors	Hyperthyroidism
Insulin-resistant diabetes	Insulin receptor	Antibody inhibits binding of insulin	Hyperglycemia, ketoacidosis
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor, decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia

Abbreviations: ANCA, antineutrophil cytoplasmic antibodies; TSH, thyroid-stimulating hormone.

presence of auto-antibodies can also represent an epiphenomen and does not necessarily indicate a pathogenetic role for a particular auto-antibody

autoimmune diseases which are primarily mediated by auto-antibodies could still originate from a primary defect in T cell tolerance

Autoimmune diseases: Examples of AID caused by self-reactive T cells

Disease	Specificity of pathogenic T cells	Human disease	Animal models
Type I (insulin-dependent) diabetes mellitus	Islet cell antigens (insulin, glutamic acid decarboxylase, others)	Yes; specificity of T cells not established	NOD mouse, BB rat, transgenic mouse models
Rheumatoid arthritis	Unknown antigen in joint synovium	Yes; specificity of T cells and role of antibody not established	Collagen-induced arthritis, others
Multiple sclerosis, experimental autoimmune encephalomyelitis	Myelin basic protein, proteolipid protein	Yes; T cells recognize myelin antigens	EAE induced by immunization with CNS myelin antigens; TCR transgenic models
Inflammatory bowel disease (Crohn's, ulcerative colitis)	Unknown	Yes	Colitis induced by depletion of regulatory T cells, knockout of IL-10
Peripheral neuritis	P2 protein of peripheral nerve myelin	Guillain-Barre syndrome	Induced by immunization with peripheral nerve myelin antigens
Autoimmune myocarditis	Myocardial proteins	Yes (post-viral myocarditis); specificity of T cells not established	Induced by immunization with myosin or infection by Coxsackie virus

: CNS, central nervous system; NOD nonobese diabetic; TCR, T cell receptor

identification of potential target auto-antigens in human AID mostly relies on extrapolation of findings from experimental models

no evidence for classification as AID!

How identify a self-directed T cell response as a pathogenetic mechanism of an AID?

- <u>experimental models</u>: immunizations with the candidate auto-antigen, transfer of pathogenic T cells to lymphopenic recipients

- <u>humans</u>: T cell infiltrates in target tissues, enhanced T cell responses to target antigens *ex vivo*, altered Th profiles (compared to healthy controls) – only circumstantial evidence!

Autoimmune diseases: Role of genetic background: MHC alleles

Associations of AID with particular HLA-DR alleles:

Disease	HLA allele	Relative risk*
Rheumatoid arthritis	DR4	4
Insulin-dependent diabetes mellitus	DR3 DR4 DR3/DR4 heterozygote	5 5–6 25
Multiple sclerosis	DR2	4
Systemic lupus erythematosus	DR2/DR3	5
Pemphigus vulgaris	DR4	14
Ankylosing spondylitis	B27	90–100

*Relative risk is defined as the probability of development of a disease in individuals with a particular HLA allele versus individuals lacking that HLA allele. The numbers given are approximations.

in experimental animal models of AID, disease susceptibility strongly depends on the genetic background

prominent role of MHCII in selection and activation of CD4 T cells

prominent role of CD4 T cells in supporting and amplifying both innate & adaptive immune responses

Cave:

- most AID are polygenic / associated with several polymorphisms
- susceptibility alleles are also found in healthy individuals
- associations may differ between ethnic groups
- linkage disequilibrium may obscure the true causal association

Autoimmune diseases: Role of genetic background: non-MHC alleles

Gene	Phenotype of mutant or knockout mouse	Mechanism of failure of tolerance	Human disease?
AIRE	Destruction of endocrine organs by antibodies, lymphocytes	Failure of central tolerance	Autoimmune polyendocrine syndrome (APS)
C4	SLE	Defective clearance of immune complexes; failure of B cell tolerance?	SLE
CTLA-4	Lymphoproliferation; T cell infiltrates in multiple organs, especially heart; lethal by 3-4 weeks	Failure of anergy in $CD4^+$ T cells	CTLA-4 polymorphisms associated with several autoimmune diseases
Fas/FasL	Anti-DNA and other autoantibodies; immune complex nephritis; arthritis; lymphoproliferation	Defective deletion of anergic self-reactive B cells; reduced deletion of mature $CD4^+$ T cells	Autoimmune lymphoproliferative syndrome (ALPS)
FoxP3	Multi-organ lymphocytic infiltrates, wasting	Deficiency of regulatory T cells	IPEX
IL-2; IL-2Ra/b	Inflammatory bowel disease; anti-erythrocyte and anti-DNA autoantibodies	Defective development, survival or function of regulatory T cells	None known
SHP-1	Multiple autoantibodies	Failure of negative regulation of B cells	None known
PTPN22	Increased lymphocyte proliferation, antibody production	Reduced inhibition by tyrosine phosphatase?	PTPN22 polymorphisms are associated with several autoimmune diseases

Abbreviations: AIRE, autoimmune regulator gene; IL-2, interleukin-2; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; SHP-1, SH2-containing phosphatase-1.

failure in central tolerance

failure in peripheral tolerance mechanisms

Autoimmune diseases: Pathogenic role of microbial infections

Potential mechanisms for pathogen-triggered induction of autoimmunity:

- molecular mimicry

AID	Cross-reacting sequences	Origin of Peptide
EAE	YGSLPQKSQRTQDEN YGCLLPRNPRTEDQN	Rat MBP *Chlamydia pneumoniae* protein
Lyme arthritis	VVLEGTLTA YVIEGTSKQ	Human LFA-1 *Borrelia burgdorferi* OspA protein
MS	VVHFFKNIV VYHFVKKHV	Human MBP EBV protein
RF	QKMRRDLEE KGLRRDLDA	Human myosin *Streptococcus* cell wall M protein

TW Mak and ME Saunders

rheumatic fever: antistreptococcal antibodies crossreact with myocardial proteins

T1 diabetes — PC2 protein of Coxsackie — GAD of islet cells

- activation of APC and “bystander” activation of self-specific T cells

  (also: upregulation of MHCII on non-APC?)

- tissue damage mediated by hypersensitivity reactions to persistent microbes may lead to the release of normally sequestered self-antigens and induction of autoimmunity

Autoimmune diseases: Pathogenetic role of microbial infections

Pro:

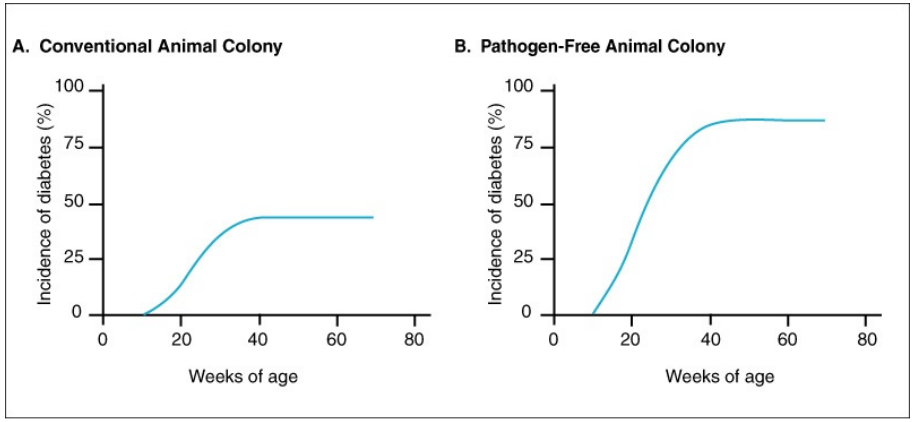
Experimental models:	Human disease:
(only some examples!) • in a spontaneous model of EAE, mice are less prone to disease when housed in a clean facility • in the NOD mouse model of diabetes disease onset (in adult mice) is accelerated after infection with rotaviruses	(only some examples!) • viral infections have been associated with flare-up of SLE and relapse of MS • viral antigens from EBV, HPV, influenza, measles virus a.o. can provoke the production of antibodies and T cells that crossreact with MBP *in vitro* • infection with enteroviruses often precedes or accompanies diabetes in children

Autoimmune diseases: Pathogenetic role of microbial infections

Contra: "Infections are common, autoimmunity is not" R. Inman, L. Albert; University of Toronto

Experimental models:

- a shift to a germ-free (GF) environment does not ameliorate disease in Aire$^{-/-}$ mice
- rheumatoid arthritis is equally inducible in germ-free rats
- SPF and GF NOD mice have a higher incidence of spontaneous diabetes

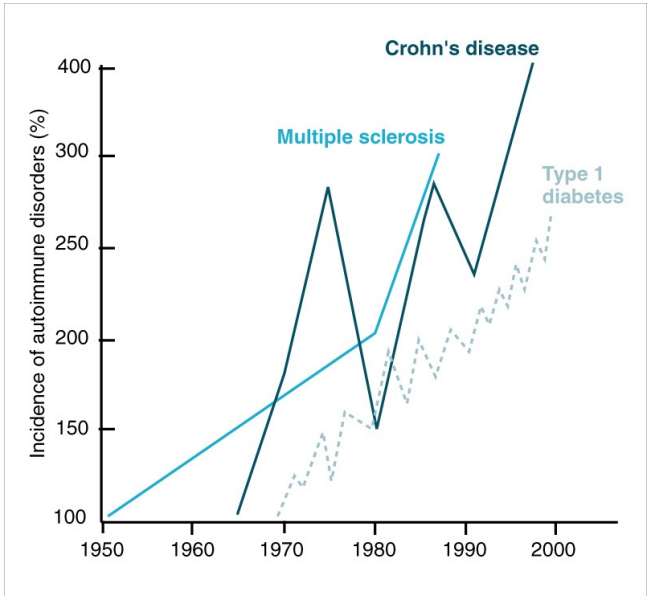


(Adapted from Bach J. F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *New England Journal of Medicine* 347, 911–920.)

can infections even protect from AID ?!

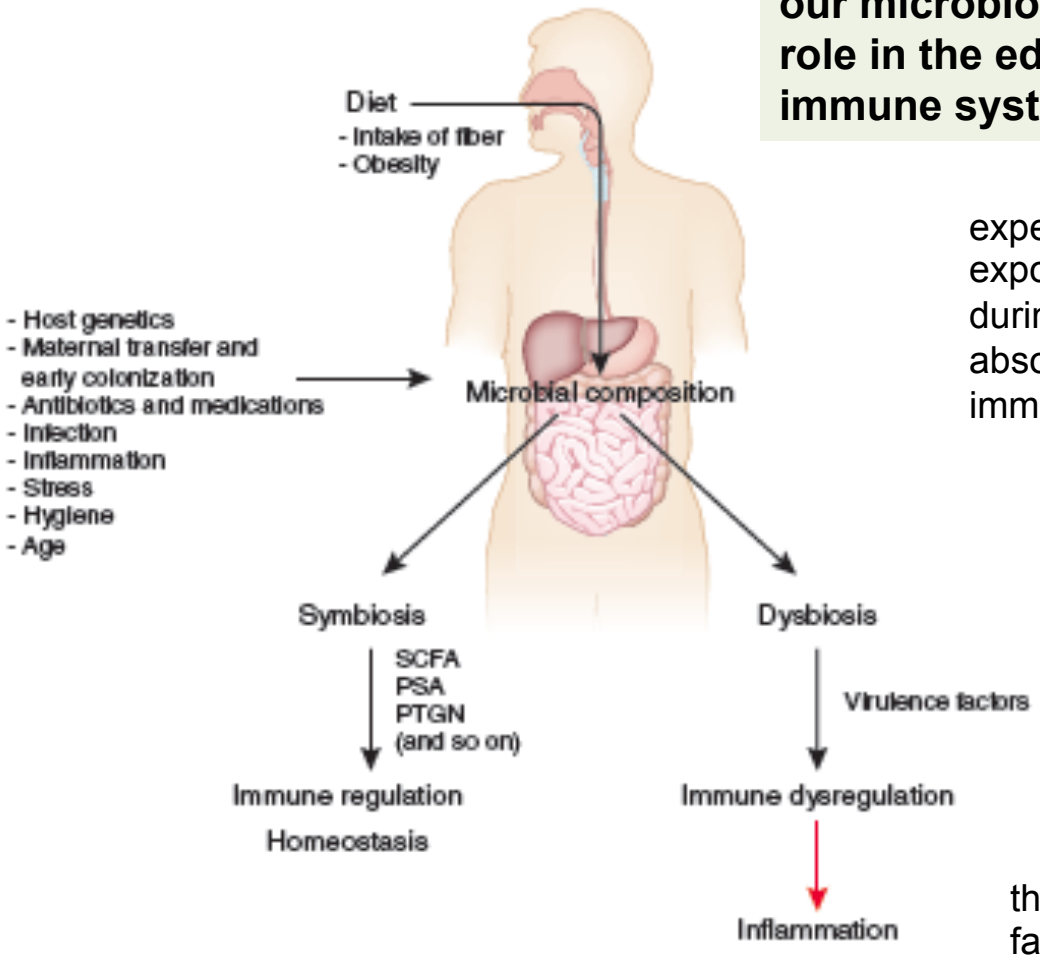
Human disease:

- for most AID, no firm causal relation-ship with a specific pathogen exists
- "hygiene hypothesis": increasing incidence of hypersensitivity diseases in developed countries



(Adapted from Bach J. F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *New England Journal of Medicine* 347, 911–920)

The hygiene hypothesis re-visited: The diet-microbiota hypothesis!



Kendle M Maslowski & Charles R Mackay

NATURE IMMUNOLOGY VOLUME 12 NUMBER 1 JANUARY 2011

our microbiota and our diet play a considerable role in the education and regulation of our immune system:

experimental models indicate that exposure to a diverse microbial population during a critical time window early in life is absolutely required to set the baseline immune regulatory state for life

human populations that consume adequate or large amounts of dietary fibers and whose microbiota is enriched in Bacteriodetes show a lower incidence of inflammatory or atopic disease

the anti-inflammatory effects of short-chain fatty acids (SCFA) and polysaccharide A (PSA) on the intestinal immune system (e.g. by expansion of Tregs or modulation of colonic epithelial cells) are now acknowledged

Autoimmune disease: where does tolerance fail ?

	Gene	Phenotype of mutant or knockout mouse	Mechanism of failure of tolerance	Human disease?
central tolerance	MHCII		failure in binding of relevant peptides for negative selection	
central tolerance	AIRE	Destruction of endocrine organs by antibodies, lymphocytes	Failure of central tolerance	Autoimmune polyendocrine syndrome (APS)
central tolerance	FoxP3	Multi-organ lymphocytic infiltrates, wasting	Deficiency of regulatory T cells	IPEX
central tolerance	IL-2; IL-2Ra/b	Inflammatory bowel disease; anti-erythrocyte and anti-DNA autoantibodies	Defective development, survival or function of regulatory T cells	None known
peripheral tolerance	C4	SLE	Defective clearance of immune complexes; failure of B cell tolerance?	SLE
peripheral tolerance	CTLA-4	Lymphoproliferation; T cell infiltrates in multiple organs, especially heart; lethal by 3-4 weeks	Failure of anergy in $CD4^+$ T cells	CTLA-4 polymorphisms associated with several autoimmune diseases
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peripheral tolerance	SHP-1	Multiple autoantibodies	Failure of negative regulation of B cells	None known
peripheral tolerance	PTPN22	Increased lymphocyte proliferation, antibody production	Reduced inhibition by tyrosine phosphatase?	PTPN22 polymorphisms are associated with several autoimmune diseases

most evidence today points to a role for defects in peripheral, rather than central tolerance mechanisms

evidence from experimental models suggests a pivotal role for T_{regs} in preventing onset of autoimmunity

in humans, most patients with AID exhibit normal numbers of functional T_{regs}

AID likely have a multifactorial aetiology with a complex interplay of genetic, microbial and even nutritional factors

Hypersensitivity diseases

Hypersensitivity:

"Excessive immune reactivity to a generelly innocuous (self- or foreign) antigen" (Tak W. Mak)

Type of hypersensitivity	Pathologic immune mechanisms	Mechanisms of tissue injury and disease
Immediate hypersensitivity: Type I	IgE antibody	Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody mediated: Type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	Opsonization and phagocytosis of cells Complement- and Fc receptor–mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, e.g., hormone receptor signaling
Immune complex mediated: Type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Complement- and Fc receptor–mediated recruitment and activation of leukocytes
T cell mediated: Type IV	1. $CD4^+$ T cells (delayed-type hypersensitivity) 2. $CD8^+$ CTLs (T cell–mediated cytolysis)	1. Macrophage activation, cytokine-mediated inflammation 2. Direct target cell killing, cytokine-mediated inflammation

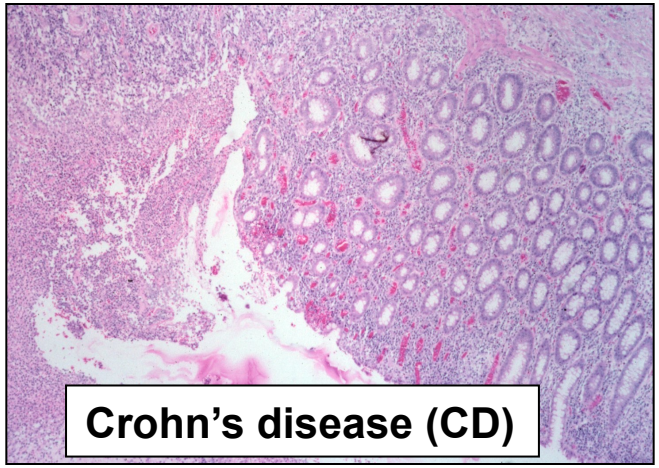
Examles for type IV	T cells directed against	tissue damage observed
• chronic DTH	conventional foreign antigen	granuloma formation
• chronic graft rejection	allo-antigen in donor tissue	destruction of donor cells, fibrosis
• autoimmunity	self-antigen in target tissue	destruction of self-cells, fibrosis

Inflammatory bowel diseases: Clinical / histological manifestation

Switzerland: ca. 1/500 inhabitants

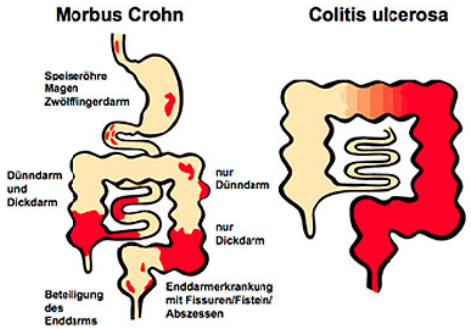
clinical symptoms: abdominal pain, diarrhea, fever, fatigue (chronic relapsing)

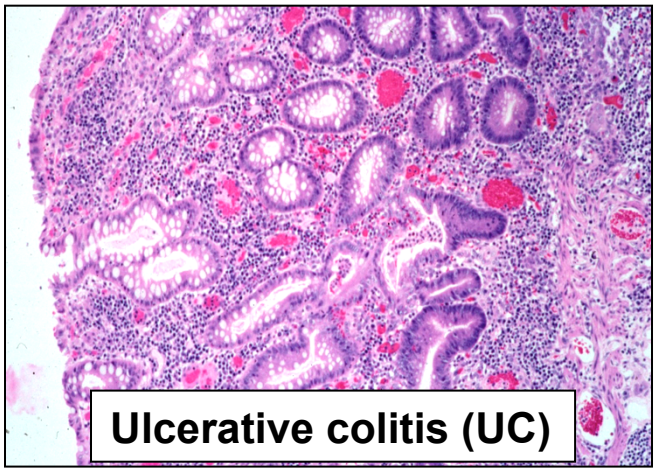
onset between 15-40 years; from first symptoms to diagnosis: > 2 years



Crohn's disease (CD)

- may affect entire gastrointestinal tract
- discontinuous, transmural, granulomatous inflammation





Ulcerative colitis (UC)

- continuous inflammation of entire colon
- crypt abscesses

treatment: anti-inflammatory / immunosuppressive drugs (5-ASA, methotrexate, steroids, anti-TNF)

Inflammatory bowel diseases (IBD): where do they belong…?

…the evolving paradigm…

IBD is a chronic immune-mediated but non-infectious disease

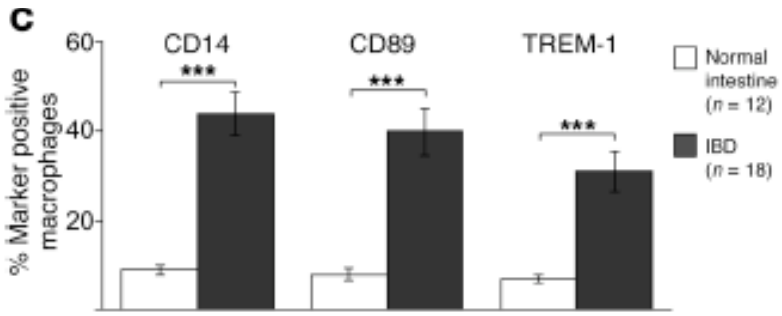
IBD is an autoimmune disease

IBD is a T cell-mediated hypersensitivity reaction against the commensal flora

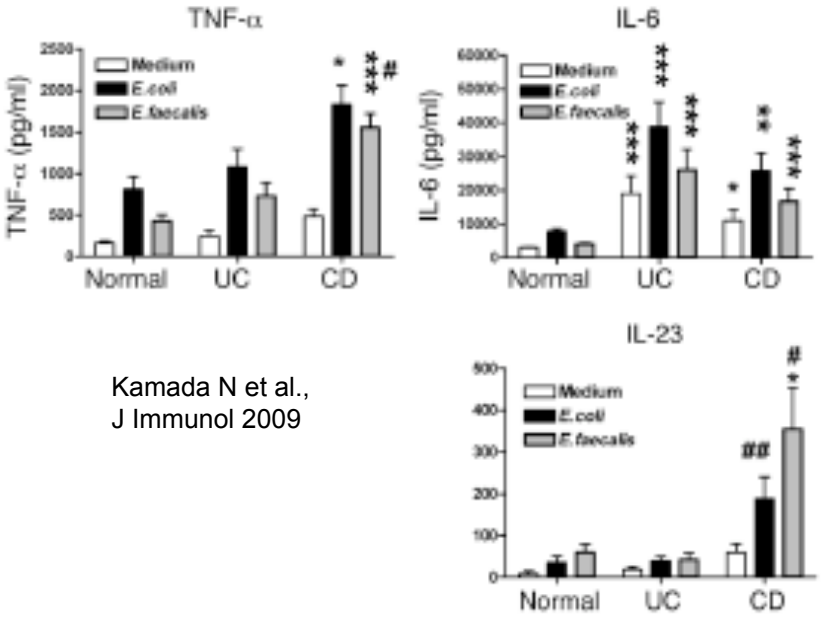
Is IBD an innate immunodeficiency disease ?

Inflammatory bowel diseases: atypical intestinal immune responses

aberrant phenotypes and functional responses of intestinal macrophages in IBD

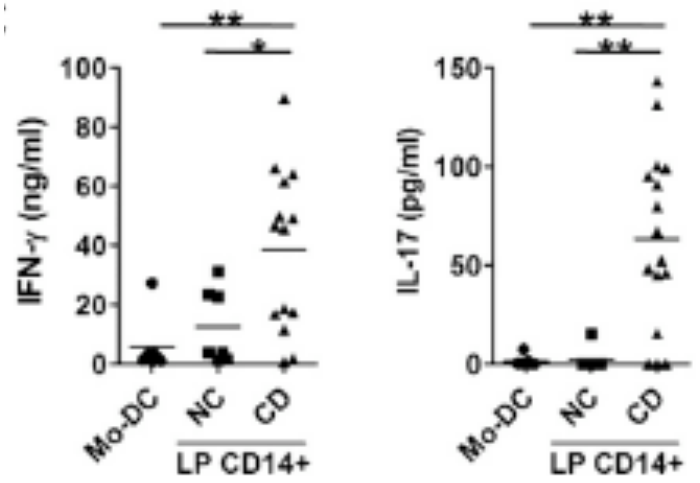


Schenk M, Mueller C et al., J Clin Invest 2007



Kamada N et al.,
J Immunol 2009

aberrant differentiation of intestinal CD4 T cells into Th1 and Th17 subsets in Crohn's disease (CD) patients



Kamada N et al., J Immunol 2009

Inflammatory bowel diseases: Role for microbial factors ?

- in human IBD patients, disease is ameliorated by antibiotic treatment or diversion of the fecal stream away from affected intestinal segments

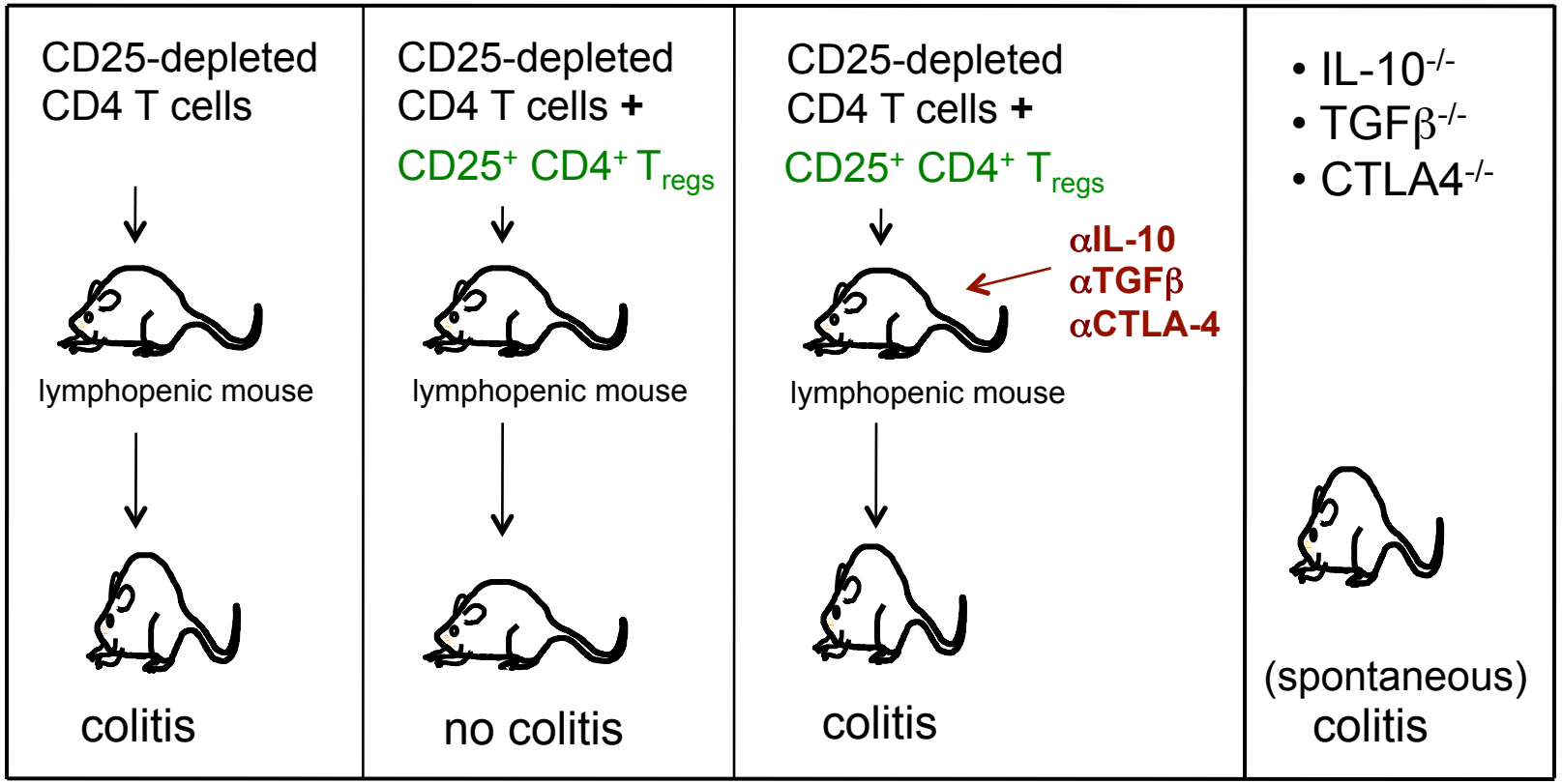
- in experimental disease models, disease cannot be induced in germ-free mice

Dysregulation of the normally tightly controlled immune response to commensal bacteria in susceptible individuals

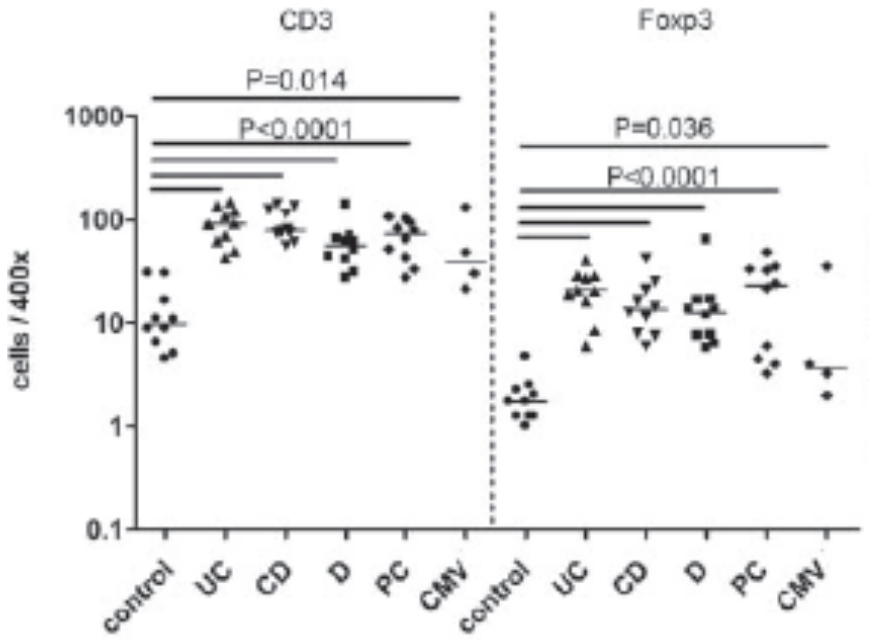
Failure in intestinal tolerance mechanisms to “foreign” ?

Inflammatory bowel diseases: Role for a defect in T_{regs} ?

- mutations in the human IL-10 receptor gene are associated with very severe early onset IBD in paediatric patients
- experimental models point to an important role for T_{reg} cells or TGFβ, IL-10 and CTLA-4 in preventing intestinal inflammation



Inflammatory bowel diseases: Role for a defect in Tregs ?

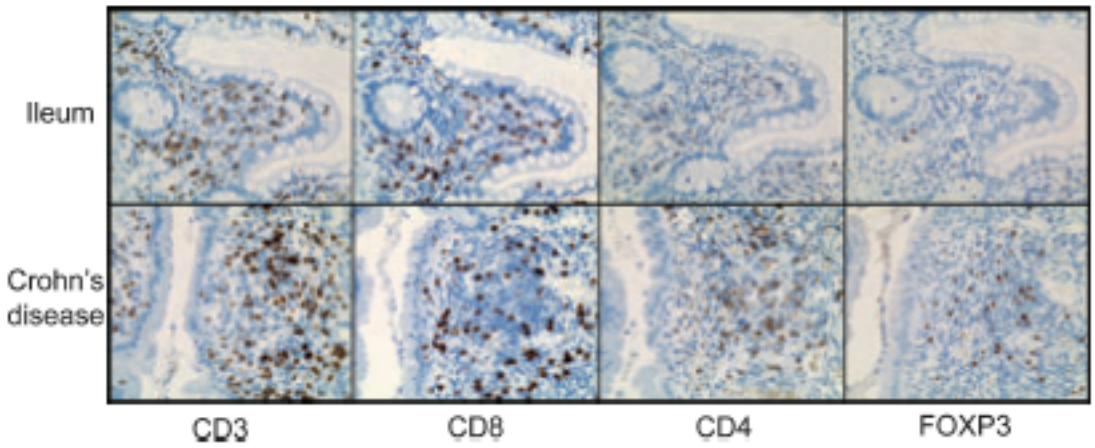


Uhlig et al., J Immunol 2006

however:

in human IBD, $CD4^+$ $CD25^+$ $Foxp3^+$ T cells are actually <u>increased</u>

no evidence for reduced production of IL-10 and TGFβ in inflamed tissues



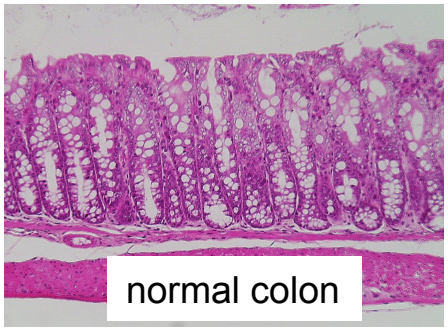
Saurer L, Mueller C, Allergy, 2009

where does tolerance fail then ?

Inflammatory bowel diseases: Role for genetic factors

Experimental models of (spontaneous) IBD:

Defective mucus layer	$Muc2^{-/-}$ mice
Impaired epithelial cell function	IEC-specific deletion of IKKγ
Aberant APC activation	$STAT3^{-/-}$ macrophages
Defect in regulatory T cells	IL-$10^{-/-}$, TGFβ$^{-/-}$, IL-$2^{-/-}$ mice



Different genetic defects, including innate immune defects, can lead to comparable clinical and histopathological signs of experimental colitis

Inflammatory bowel diseases: Role for genetic factors

susceptibility allelles Crohn's disease

gene	normal function
NOD2/ CARD15	intracellular PRR, recognition of bacterial peptidoglycan
IL-23R	subunit of IL-23 receptor, Th17 differentiation
IL-12B	p40 subunit of IL-23 and IL-12, Th1 differentiation
STAT3	various, involvement in Th17 differentiation
ATG16L1	autophagy

human susceptibility loci support the notion that IBD may initially originate from an aberrant innate immune response

Inflammatory bowel diseases: Role for genetic factors

A closer look at the functions of some of the disease-associated alleles reveals a potential innate immune defect rather than hyperresponsive state

NOD2

disease-associated polymorphism reduces function of NOD2:
- impaired handling of bacteria by macrophages?
- decreased antimicrobial peptide expression by epithelial cells?

IL-23R

- impaired IL-22 production in innate lymphoid cells?
- decreased antimicrobial peptide expression by epithelial cells?

ATG16L1

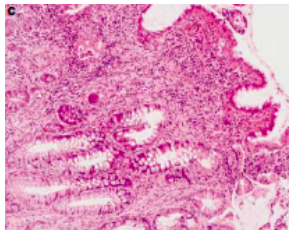
- impaired anti-bacterial autophagy?
- impaired Paneth cell exocytosis?

increased penetration of bacteria, uncontrolled activation of compensatory adaptive responses?

Crohn's disease: An innate immune deficiency disease ?

Circumstantial evidence:

IBD in ≈ 30% of patients with chronic granulomatous disease (NADPH defect!)

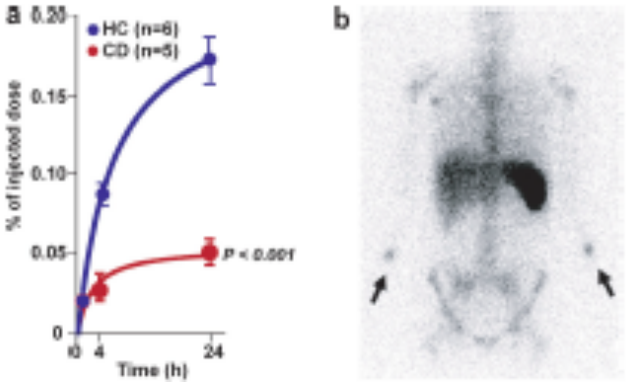


Inflammatory Bowel Disease in CGD Reproduces the Clinicopathological Features of Crohn's Disease

Daniel J.B. Marks, PhD[1,4], Kara Miyagi, MB BS, BSc[1,4], Farooq Z. Rahman, MRCP[1], Marco Novelli, PhD[2], Stuart L. Bloom, DM, FRCP[3] and Anthony W. Segal, PhD, FRS[1]

The American Journal of GASTROENTEROLOGY

Impaired neutrophil accumulation in CD patients



i.v. injection of ^{111}Indium-labeled neutrophils
simultaneous s.c. injection of killed E. Coli

JEM

Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease

Andrew M. Smith,[1] Farooq Z. Rahman,[1] Bu'Hussain Hayee,[1] Simon J. Graham,[4] Daniel J.B. Marks,[1] Gavin W. Sewell,[1] Christine D. Palmer,[1] Jonathan Wilde,[4] Brian M.J. Foxwell,[5] Israel S. Gloger,[4] Trevor Sweeting,[2] Mark Marsh[3], Ann P. Walker,[1] Stuart L. Bloom,[6] and Anthony W. Segal[1]

JEM VOL. 206, August 31, 2009

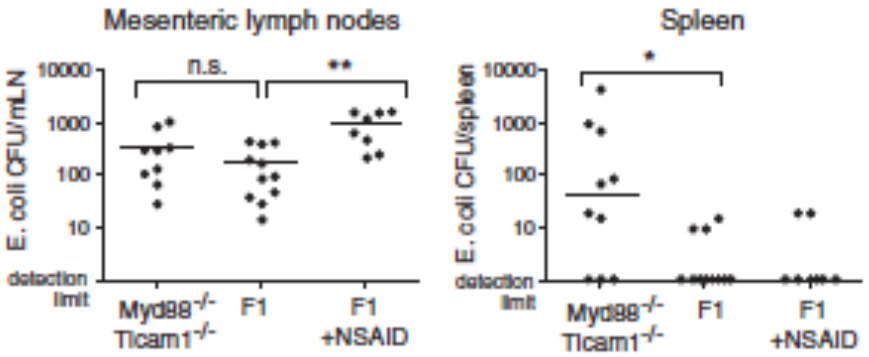
Crohn's disease: an innate immune deficiency disease ?

More circumstantial evidence:

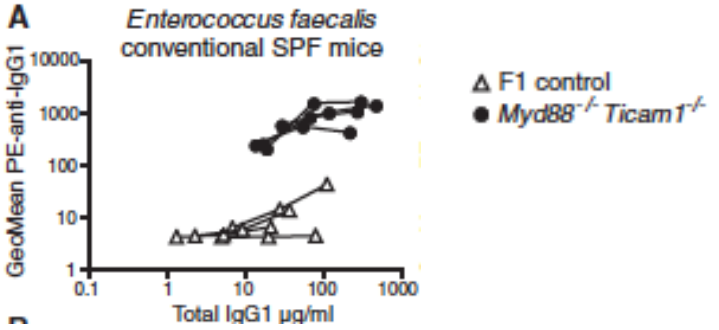
31 JULY 2009 VOL 325 SCIENCE

Innate and Adaptive Immunity Cooperate Flexibly to Maintain Host-Microbiota Mutualism

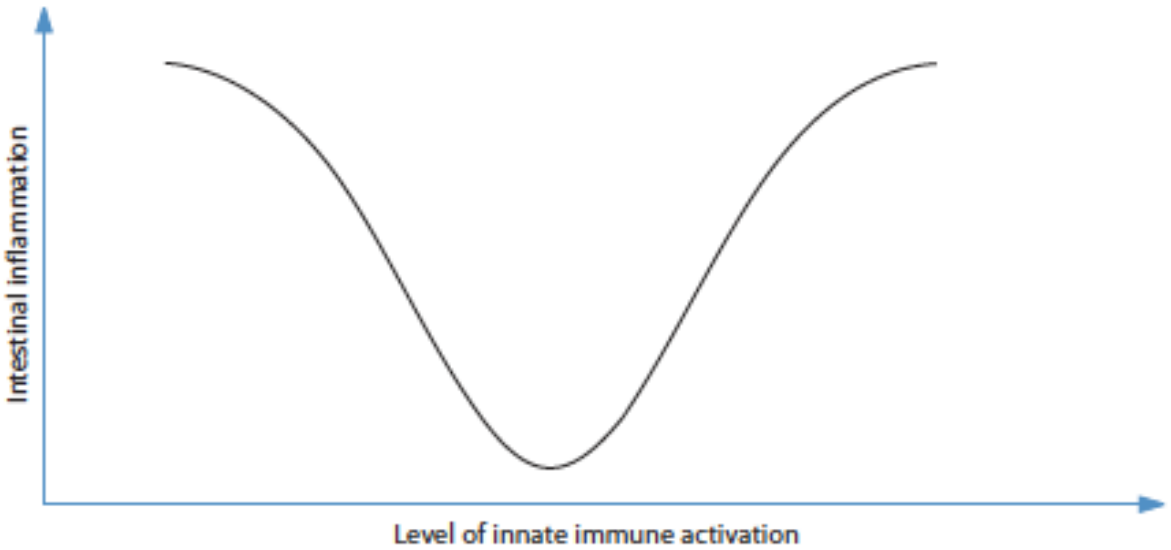
Emma Slack,[1,5]* Siegfried Hapfelmeier,[1,5] Bärbel Stecher,[2] Yuliya Velykoredko,[1] Maaike Stoel,[1] Melissa A. E. Lawson,[1] Markus B. Geuking,[1] Bruce Beutler,[3] Thomas F. Tedder,[4] Wolf-Dietrich Hardt,[2] Premysl Bercik,[1] Elena F. Verdu,[1] Kathy D. McCoy,[1] Andrew J. Macpherson[1,5]*



Mice deficient in Myd88 exhibit translocation of commensal bacteria to the spleen and loss of systemic ignorance to the commensal flora (priming of systemic adaptive immune responses)



Balanced innate immune activation maintains intestinal homeostasis



Hyporesponsive	Homeostasis	Hyperresponsive
↓ IEC integrity + proliferation	Mucus, AMP, sIgA and IEC barrier	↑ Myeloid MAMP detection
↓ Anti-microbial peptide production	PRR compartmentalization	↑ Pro-inflammatory cytokines + chemokines
↓ Luminal sampling	Tolerogenic DCs	↓ Tolerogenic pathways
↓ Pathogen/parasite clearance	Innate regulatory cytokines	↑ Leucocyte accumulation + activation
↓ PRR activation of IEC		↑ IEC proliferation

Harrison OJ, Maloy KJ
J Innate Immunity 2011

Sum-up: Immunological tolerance

- immunological tolerance (in its wider context – including tolerance to foreign) is involved in many areas of clinical immunology, including immunity to infectious agents, allergology, tumor immunology, vaccinology, autoimmunity, hypersensitivity diseases, transplantation immunology,…

- without the use of experimental models, our understanding of tolerance and breakdown of tolerance would still be in its infancy

- emerging data from clinical investigations is in line with many findings from experimental models (or vice versa) but discrepancies exist!

- future research directed at a better understanding of tolerance and breakdown of tolerance must continue to take into consideration aspects from both experimental and clinical immunology

- …replacing paradigms requires open minds ☺

"I expect you all to be independent, innovative, critical thinkers who will do exactly as I say!"