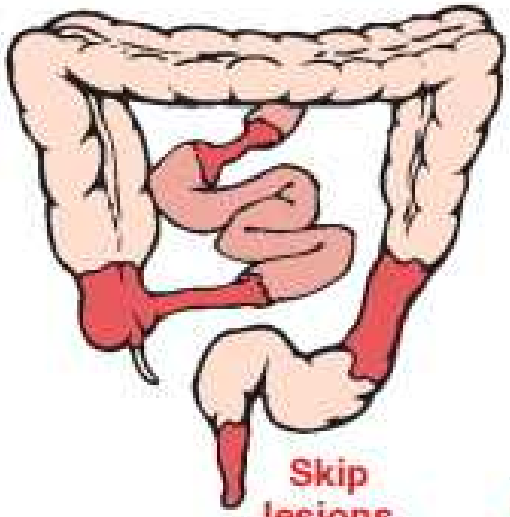
Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic condition resulting from inappropriate mucosal immune activation. The two disorders that comprise IBD are Crohn disease and ulcerative colitis. Descriptions of ulcerative colitis and Crohn disease date back to antiquity and at least the sixteenth century, respectively, but it took modern bacteriologic techniques to exclude conventional infectious etiologies for these diseases. However, commensal bacteria normally present in the intestinal lumen are probably involved in IBD pathogenesis.

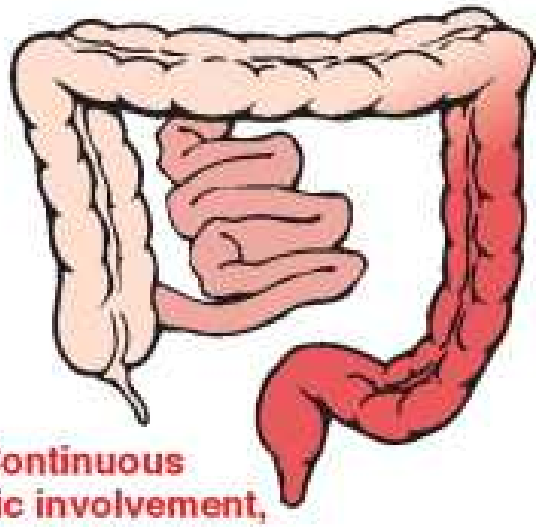
The distinction between ulcerative colitis and Crohn disease is based, in large part, on the distribution of affected sites and the morphologic expression of disease at those sites. Ulcerative colitis is a severe ulcerating inflammatory disease that is limited to the colon and rectum and extends only into the mucosa and submucosa. In contrast, Crohn disease, which has also been referred to as regional enteritis (because of frequent ileal involvement) may involve any area of the GI tract and is typically transmural.

CROHN DISEASE

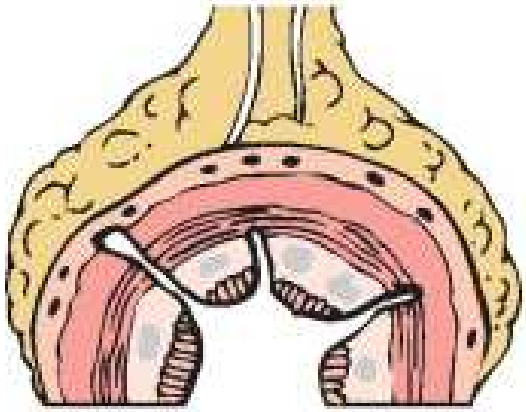


Skip lesions

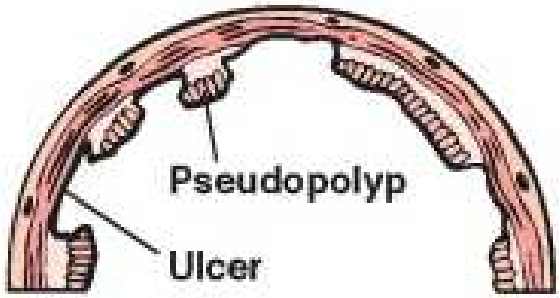
ULCERATIVE COLITIS



Continuous colonic involvement, beginning in rectum



Transmural inflammation
Ulcerations
Fissures



Features That Differ between Crohn Disease and Ulcerative Colitis

Feature	Crohn Disease	Ulcerative Colitis
MACROSCOPIC		
Bowel region	Ileum ± colon	Colon only
Distribution	Skip lesions	Diffuse
Stricture	Yes	Rare
Wall appearance	Thick	Thin
MICROSCOPIC		
Inflammation	Transmural	Limited to mucosa
Pseudopolyps	Moderate	Marked
Ulcers	Deep, knife-like	Superficial, broad-based
Lymphoid reaction	Marked	Moderate
Fibrosis	Marked	Mild to none
Serositis	Marked	Mild to none
Granulomas	Yes (~35%)	No
Fistulae/sinuses	Yes	No
CLINICAL		
Perianal fistula	Yes (in colonic disease)	No
Fat/vitamin malabsorption	Yes	No
Malignant potential	With colonic involvement	Yes
Recurrence after surgery	Common	No
Toxic megacolon	No	Yes

Note: All features may not be present in a single case.

Epidemiology

Both **Crohn disease** and **ulcerative colitis** are more **common** in **females** and frequently present in the teens and early 20s. In Western industrialized nations IBD is most **common among Caucasians** and, in the United States, occurs **3 to 5 times more often among eastern European (Ashkenazi) Jews**. This is at least partly due to genetic factors. The **geographic distribution** of IBD is highly variable, but it is **most common in North America, northern Europe, and Australia**. However, IBD incidence worldwide is on the rise, and it is becoming more common in regions such as Africa, South America, and Asia, where the prevalence was historically low. The *hygiene hypothesis* suggests that this increasing incidence may be related to **improved food storage conditions and decreased food contamination**. This hypothesis suggests that reduced frequency of enteric infections has resulted in **inadequate development of regulatory processes to limit mucosal immune responses**, allowing pathogens that should cause self-limited disease to trigger overwhelming immune responses and chronic inflammatory disease in susceptible hosts. Although many details to support this hypothesis are lacking, the observation that **helminth infection**, which is endemic in regions where IBD incidence is low, can prevent IBD development in animal models and reduce disease in some patients lends support to this idea. The observation that an episode of acute infectious gastroenteritis may precede onset of IBD in some individuals is also consistent with the hygiene hypothesis.

Robbins and Cotran “Pathologic Basis of Disease” 8°Edition

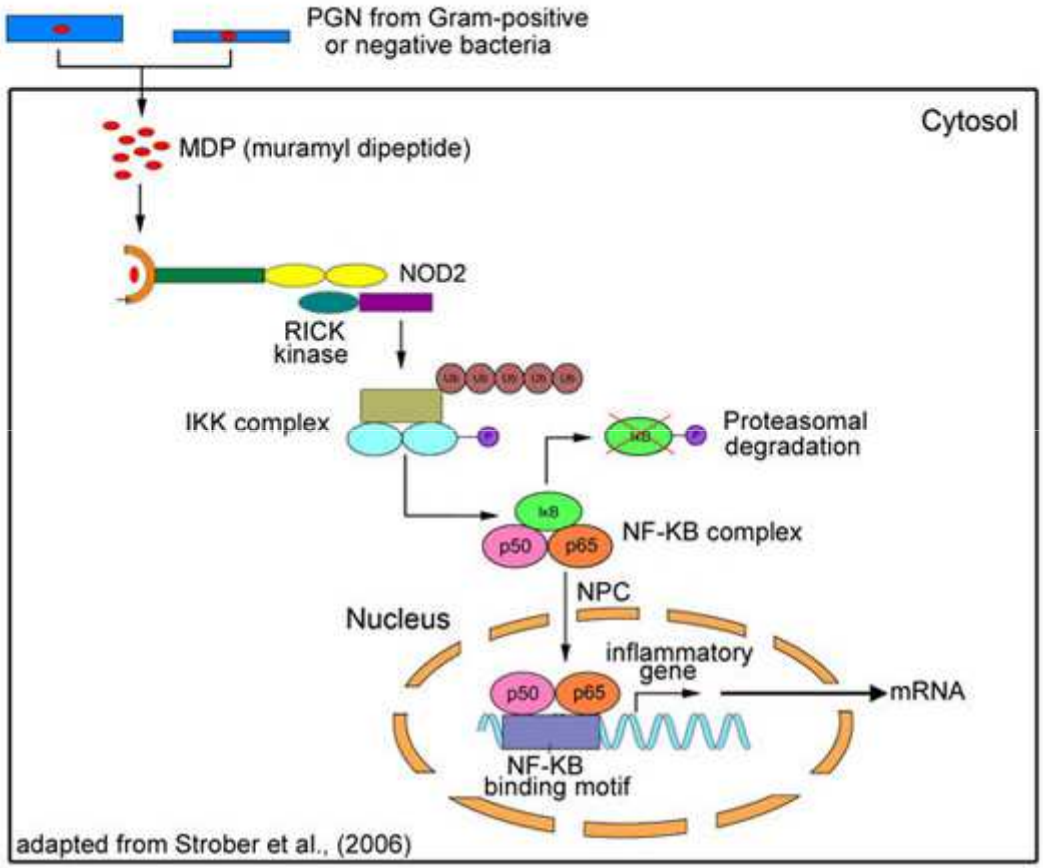
Pathogenesis

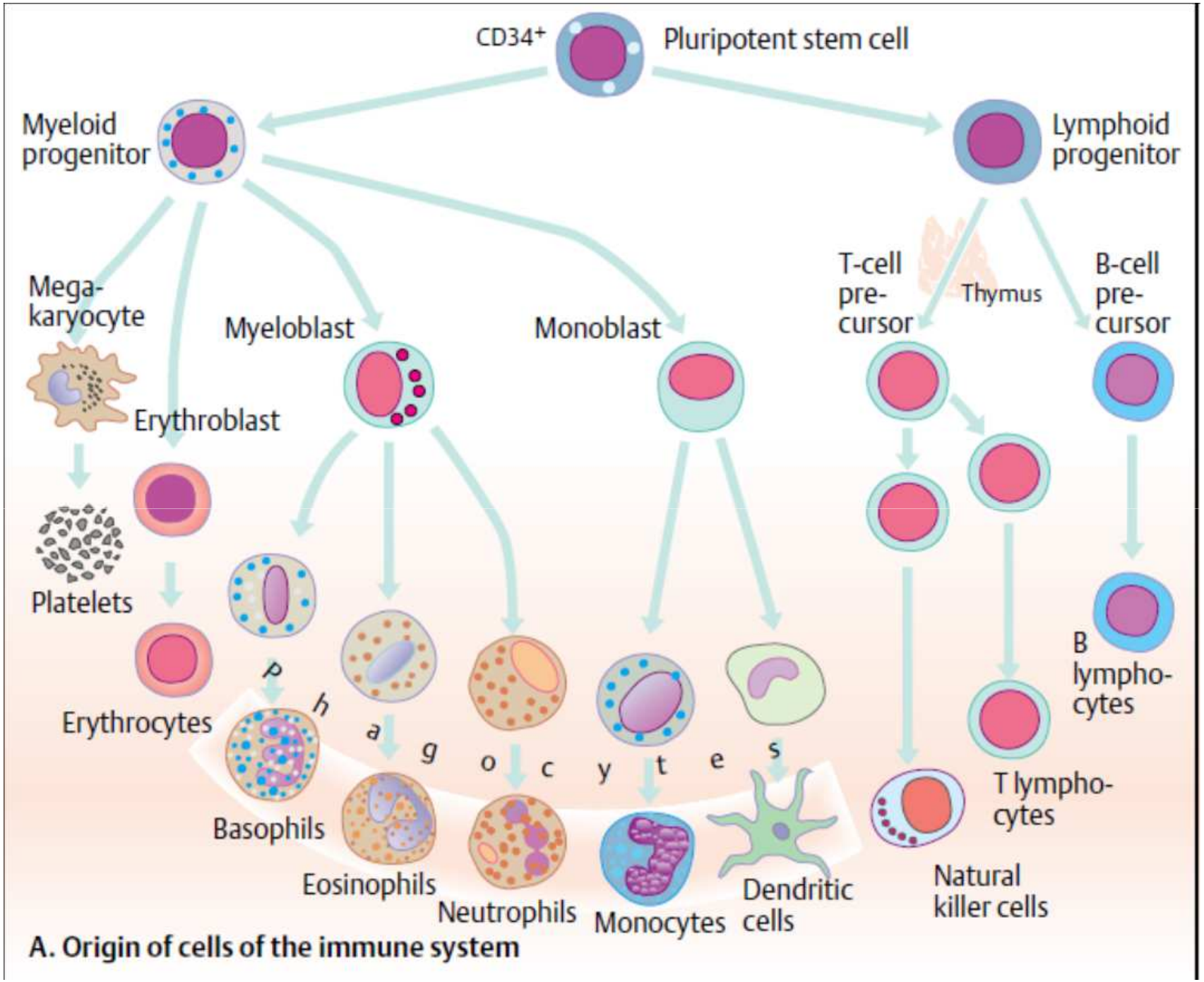
IBD is an idiopathic disorder and the responsible processes are only beginning to be understood. Although there is limited epidemiologic association of IBD with autoimmunity, neither Crohn disease nor ulcerative colitis is thought to be an autoimmune disease. Rather, most investigators believe that the two diseases result from a combination of defects in host interactions with intestinal microbiota, intestinal epithelial dysfunction, and aberrant mucosal immune responses. This view is supported by epidemiologic, genetic, and clinical studies as well as data from laboratory models of IBD.

Genetics

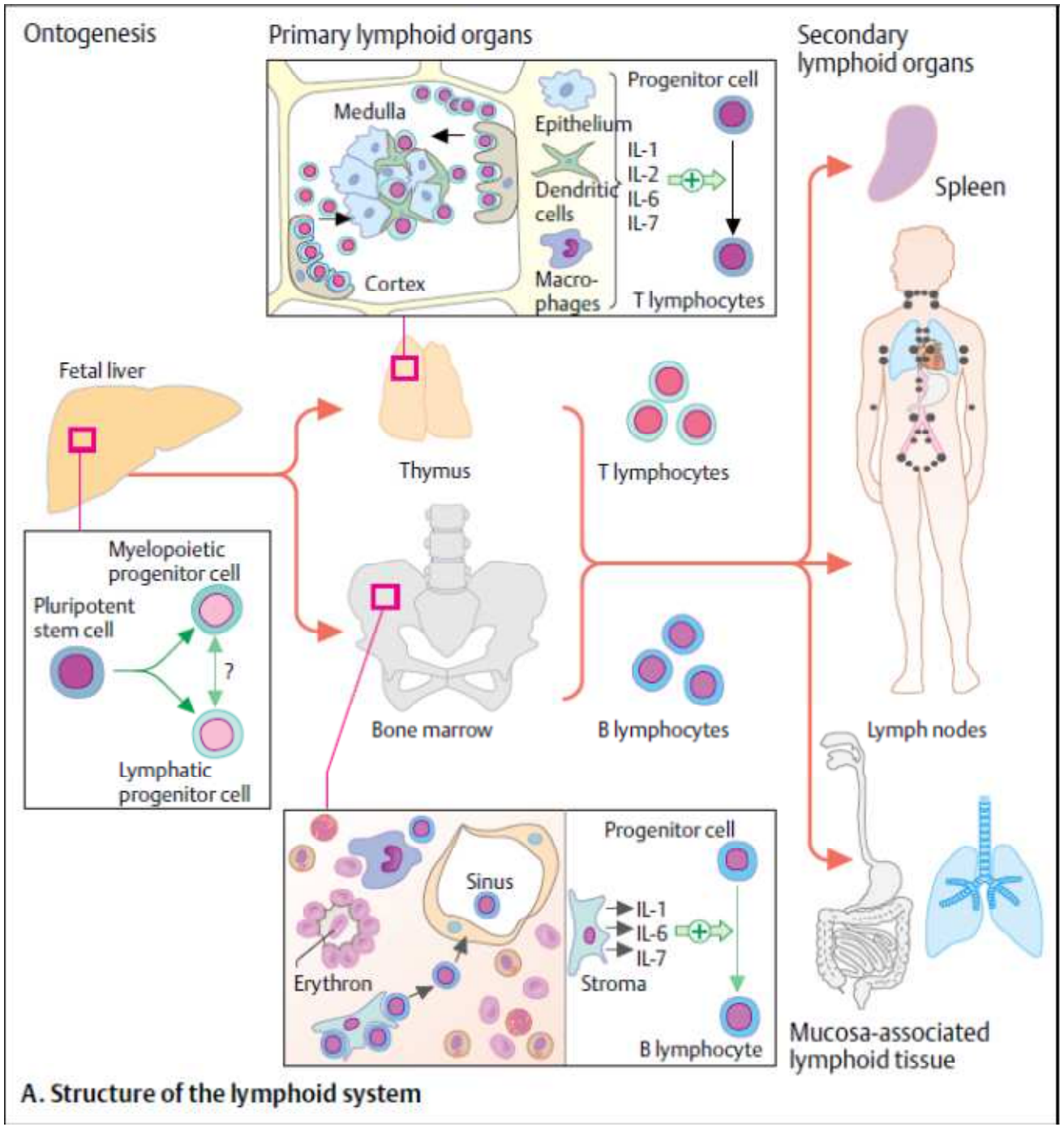
Genetic factors contribute to IBD. Risk of disease is increased when there is an affected family member and, in Crohn disease, the **concordance rate for monozygotic twins** is **approximately 50%**. The same factors may also **contribute to disease phenotype**, because twins affected by Crohn disease tend to present within 2 years of each other and develop disease in similar regions of the GI tract. The **concordance** of monozygotic twins **for ulcerative colitis is only 16%**, suggesting that genetic factors are less dominant than in Crohn disease. Concordance for dizygotic twins is less than 10% for both Crohn disease and ulcerative colitis.

Molecular linkage analyses of affected families have identified **NOD2 (nucleotide oligomerization binding domain 2)** as a susceptibility gene in Crohn disease. Specific NOD2 polymorphisms confer at least a four-fold increase in Crohn disease risk among Caucasians of European ancestry. NOD2 encodes a protein that binds to intracellular bacterial peptidoglycans and subsequently activates NF-κB. It has been postulated that disease-associated NOD2 variants are less effective at recognizing and combating luminal microbes, which are then able to enter the lamina propria and trigger inflammatory reactions. Other data suggest that NOD2 may regulate immune responses to prevent excessive activation by luminal microbes. Whatever the mechanism by which NOD2 polymorphisms contribute to Crohn disease pathogenesis, it should be remembered that **fewer than 10% of individuals carrying NOD2 mutations develop disease**. Furthermore, NOD2 mutations are uncommon in African and Asian Crohn disease patients. Thus, defective NOD2 signaling is only one of many genetic factors that contribute to Crohn disease pathogenesis.


PGN from Gram-positive
or negative bacteria
Cytosol
MDP (muramyl dipeptide)
NOD2
RICK
kinase
IKK complex
Proteasomal
degradation
IκB
p50
p65
NF-KB complex
NPC
Nucleus
inflammatory
gene
mRNA
NF-KB
binding motif
adapted from Strober et al., (2006)



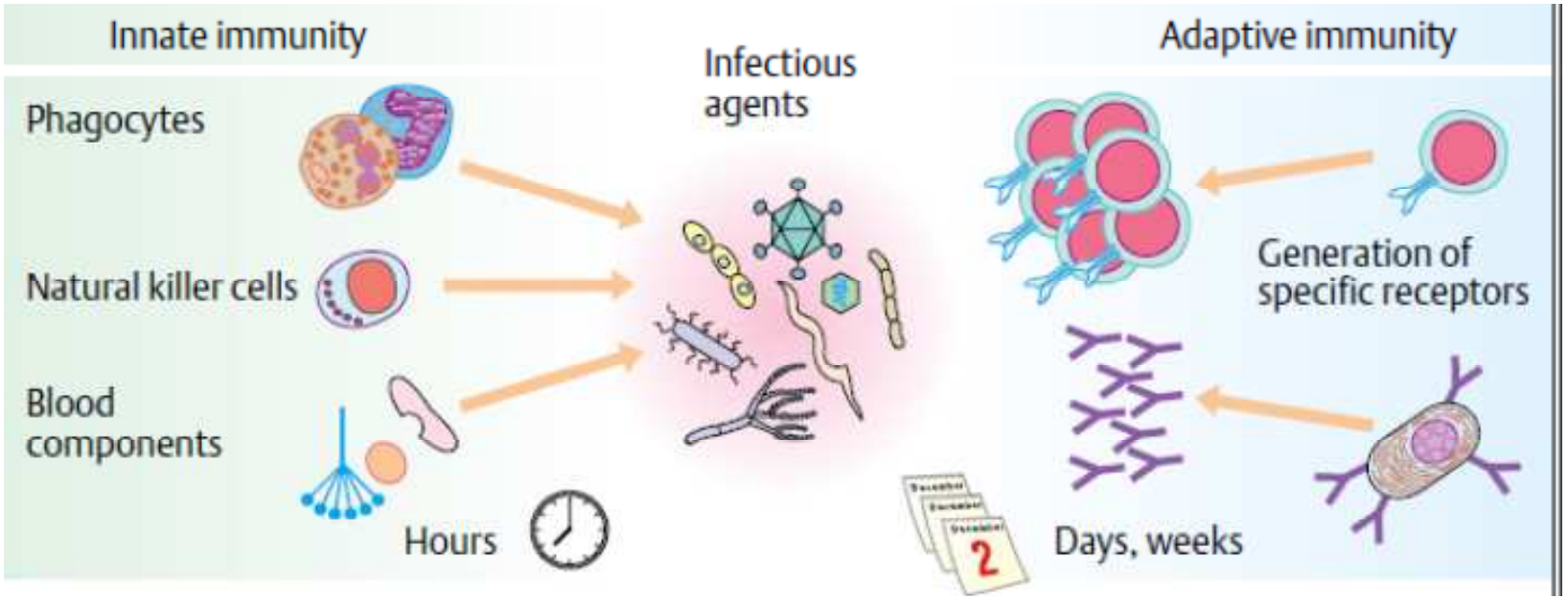
A. Origin of cells of the immune system



A. Structure of the lymphoid system

Immunità non specifica (innata)

L'immunità aspecifica o innata costituisce la branca evolutivamente più antica e l'impalcatura fondamentale del sistema immunitario. Essa infatti non solo si configura come prima linea di difesa dell'organismo contro il non-self, ma funge anche da innesco e da "forza lavoro" ausiliaria per la risposta immunitaria specifica coordinata dai linfociti T-helper.



Immunità specifica (acquisita)

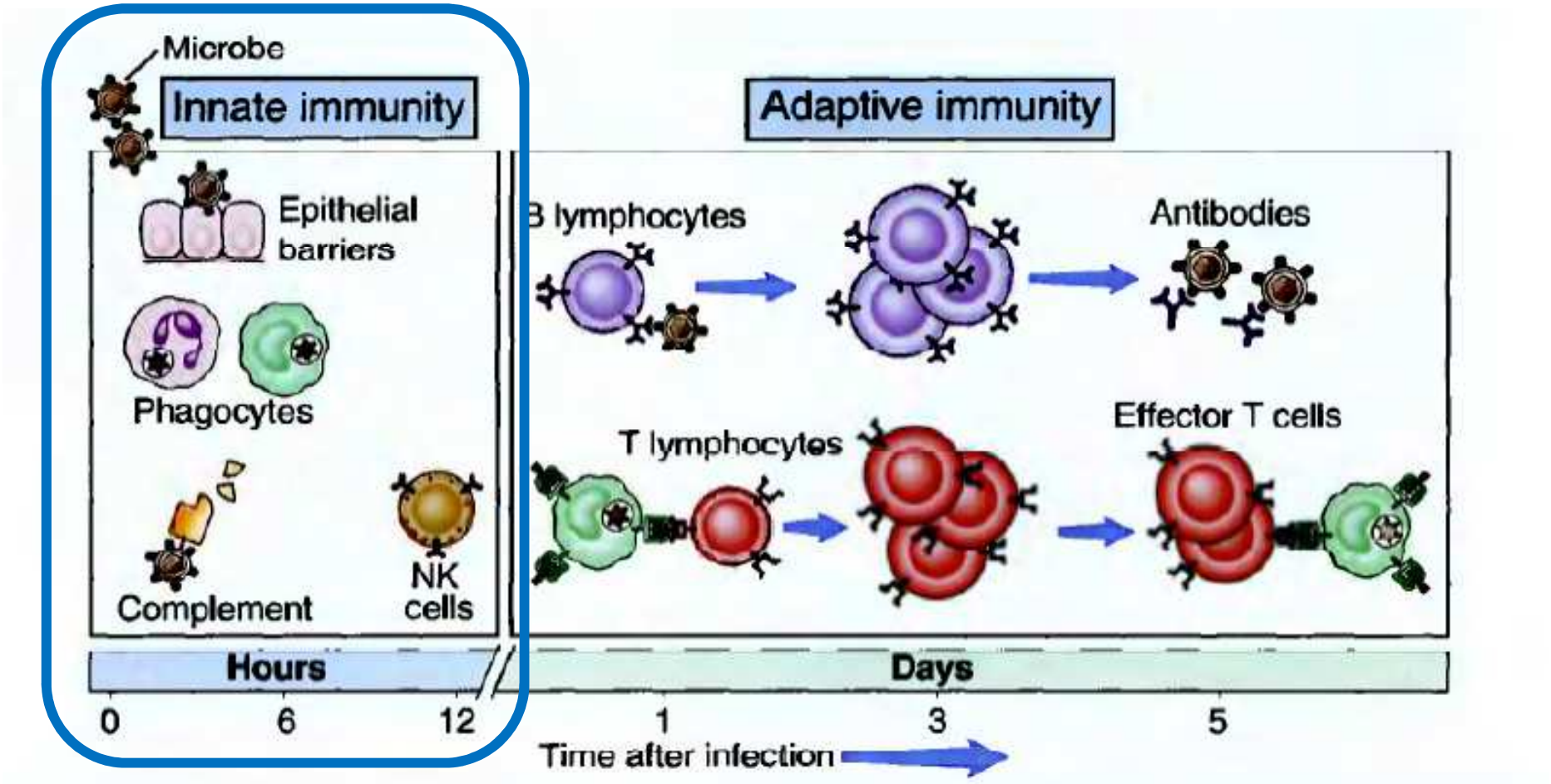
L'immunità specifica è costituita prevalentemente da cellule della linea linfoide (della serie T e B) e da cellule accessorie. I linfociti T si suddividono in linfociti T helper CD4+ e linfociti T citotossici (CTL) CD8+. La funzione effettrice dei primi è quella di coordinare il complesso della risposta immune attivando linfociti CD8+ e macrofagi (T-helper 1) o linfociti B (T-helper 2) e di sostenere il processo infiammatorio. Tale attività è svolta attraverso interazioni cellula-cellula o mediante rilascio di particolari fattori solubili detti citochine. La funzione effettrice dei linfociti CD8+ è quella di lisare le cellule infette grazie alla produzione delle linfochine. I linfociti B attivati si specializzano invece in cellule secernenti anticorpi (plasmacellulle). Le cellule accessorie sono le cellule reclutate dal compartimento innato del sistema immunitario. A differenza dell'immunità aspecifica o innata l'immunità specifica o acquisita è stata selezionata dall'evoluzione per la sua capacità di adattarsi dinamicamente alla variabilità di agenti ambientali riconosciuti come un pericolo per l'organismo. Tale variabilità è ovviamente una caratteristica peculiare di molti microrganismi infettivi in continua co-evoluzione con il sistema immunitario che cerca di distruggerli.

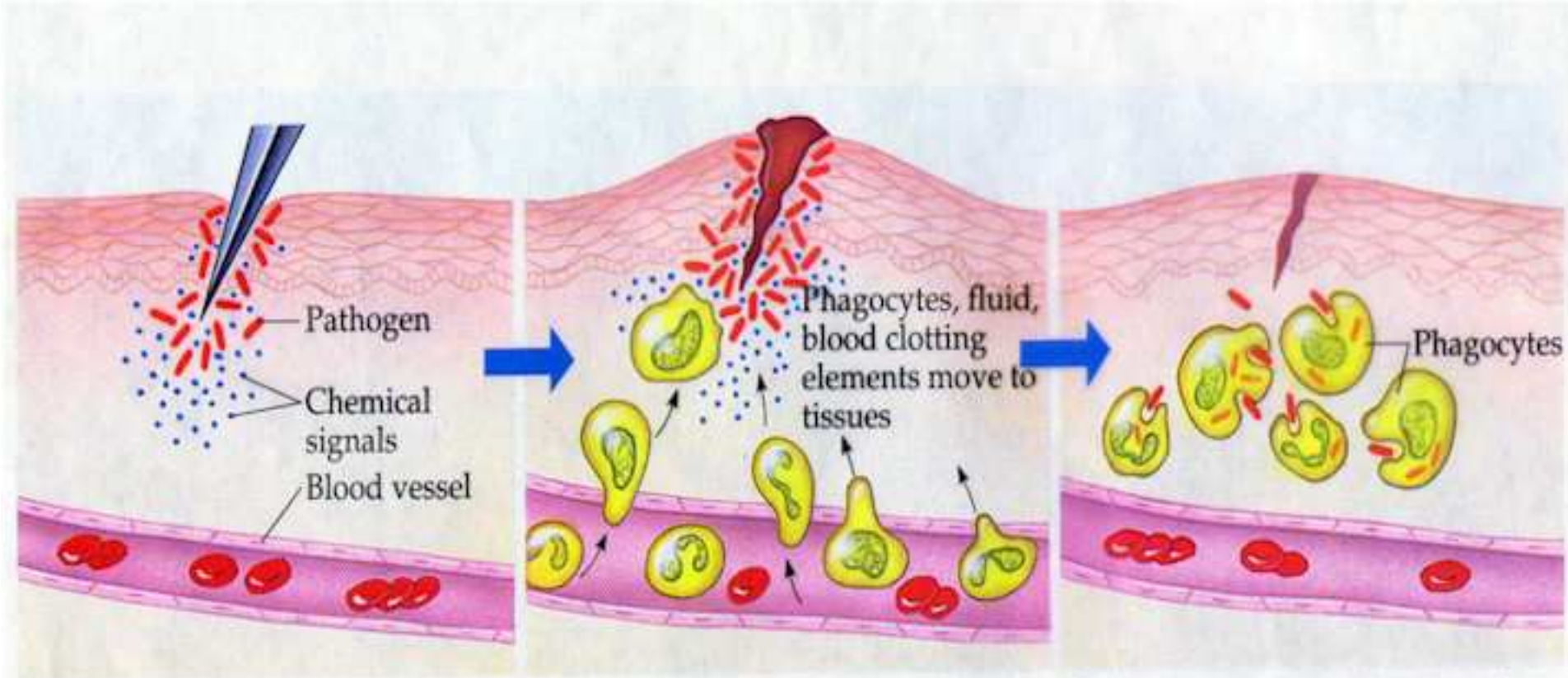
Immunità non specifica (innata)

Barriere chimico-fisiche: rivestimento cutaneo ed epiteli che rivestono le mucose, lisozima, temperatura corporea

Componenti cellulari: cellule di natura fagocitaria (macrofagi e granulociti), cellule endoteliali, mastociti, piastrine (entrambe definite cellule ausiliarie) ed NK (natural killer)

Molecole circolanti: proteine del complemento (capaci di mediare difesa dell'ospite, mediante lisi ed opsonizzazione), citochine (interferoni, IL-1 e TNF) deputate alla regolazione della risposta infiammatoria

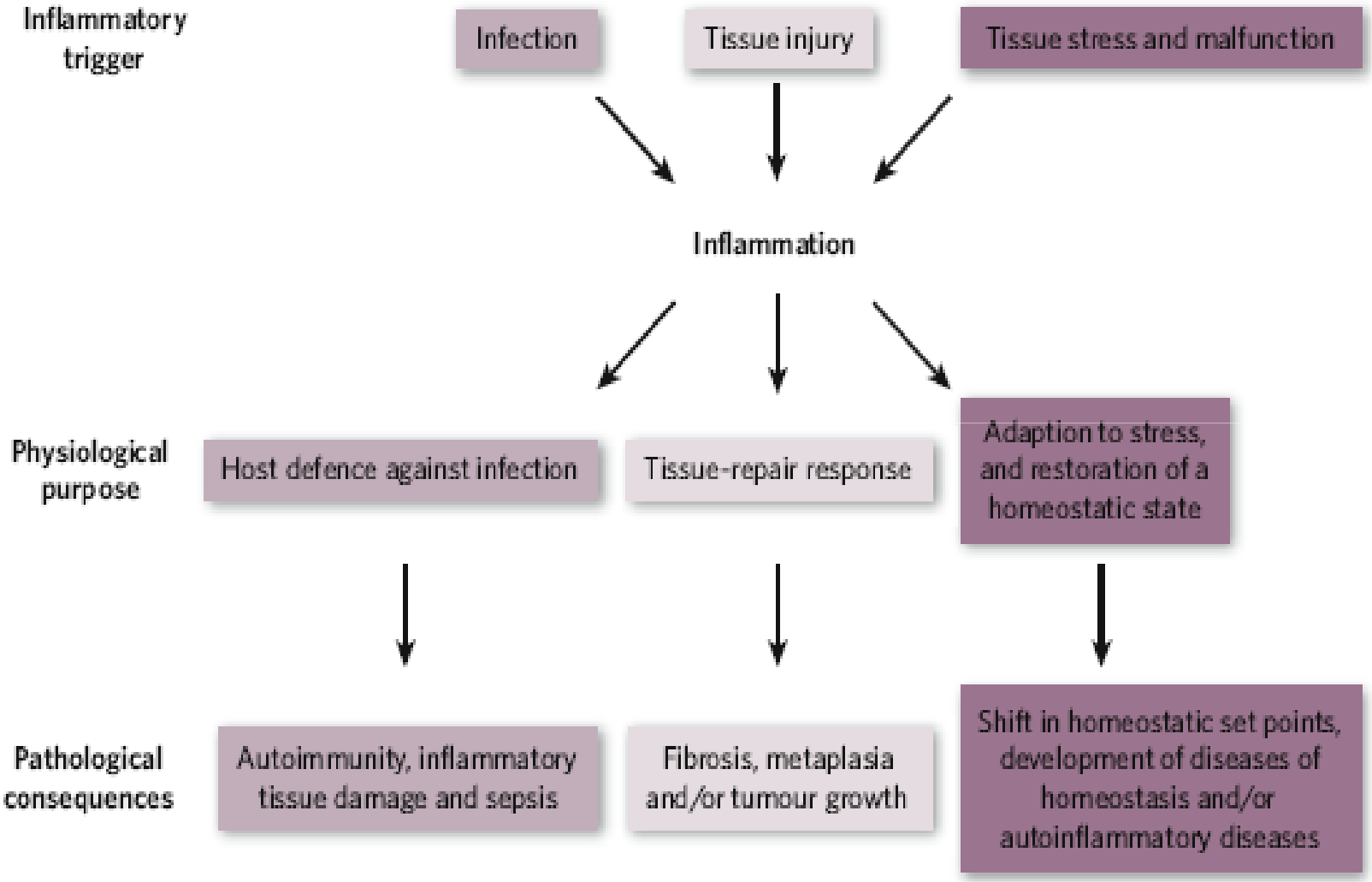


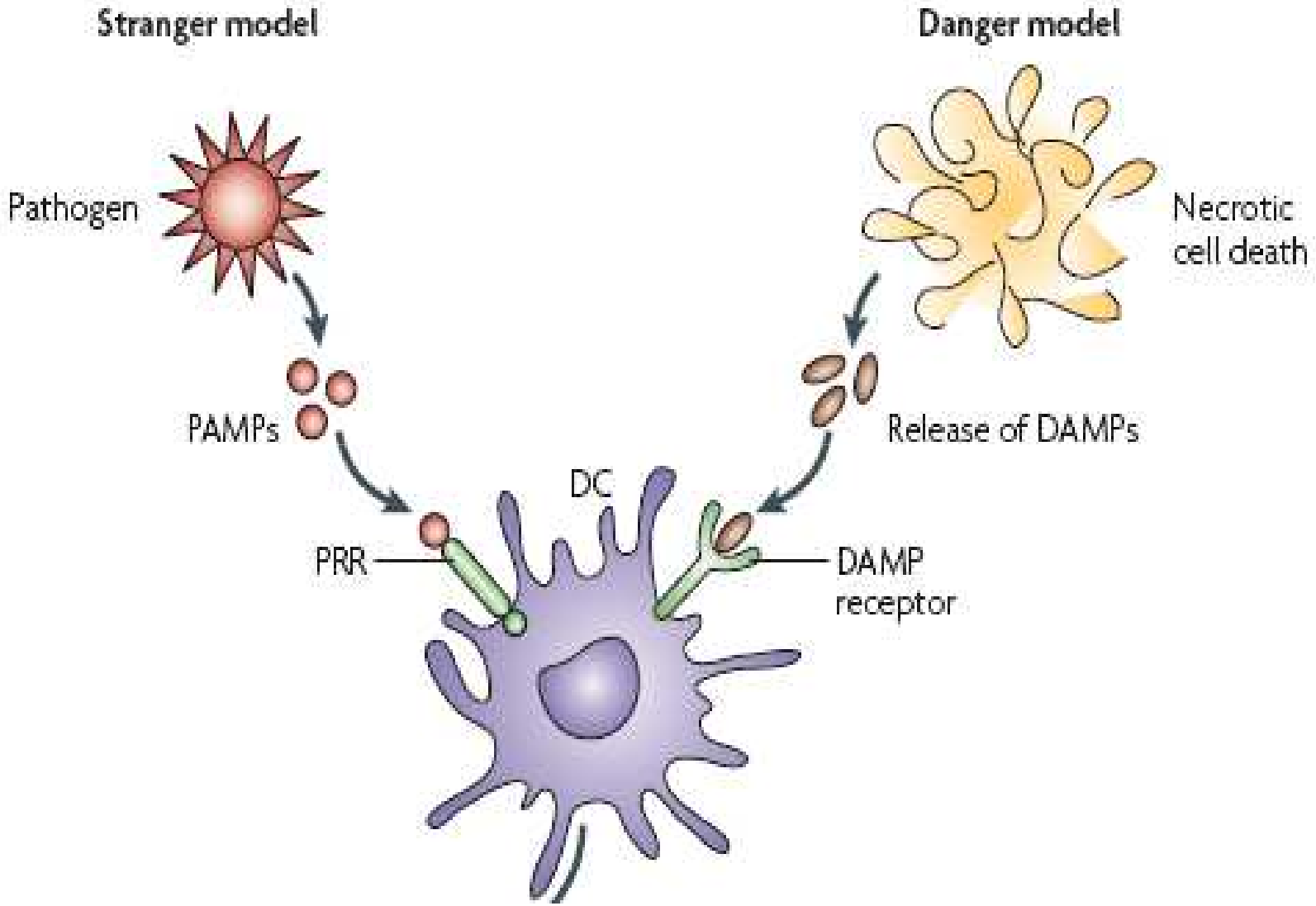


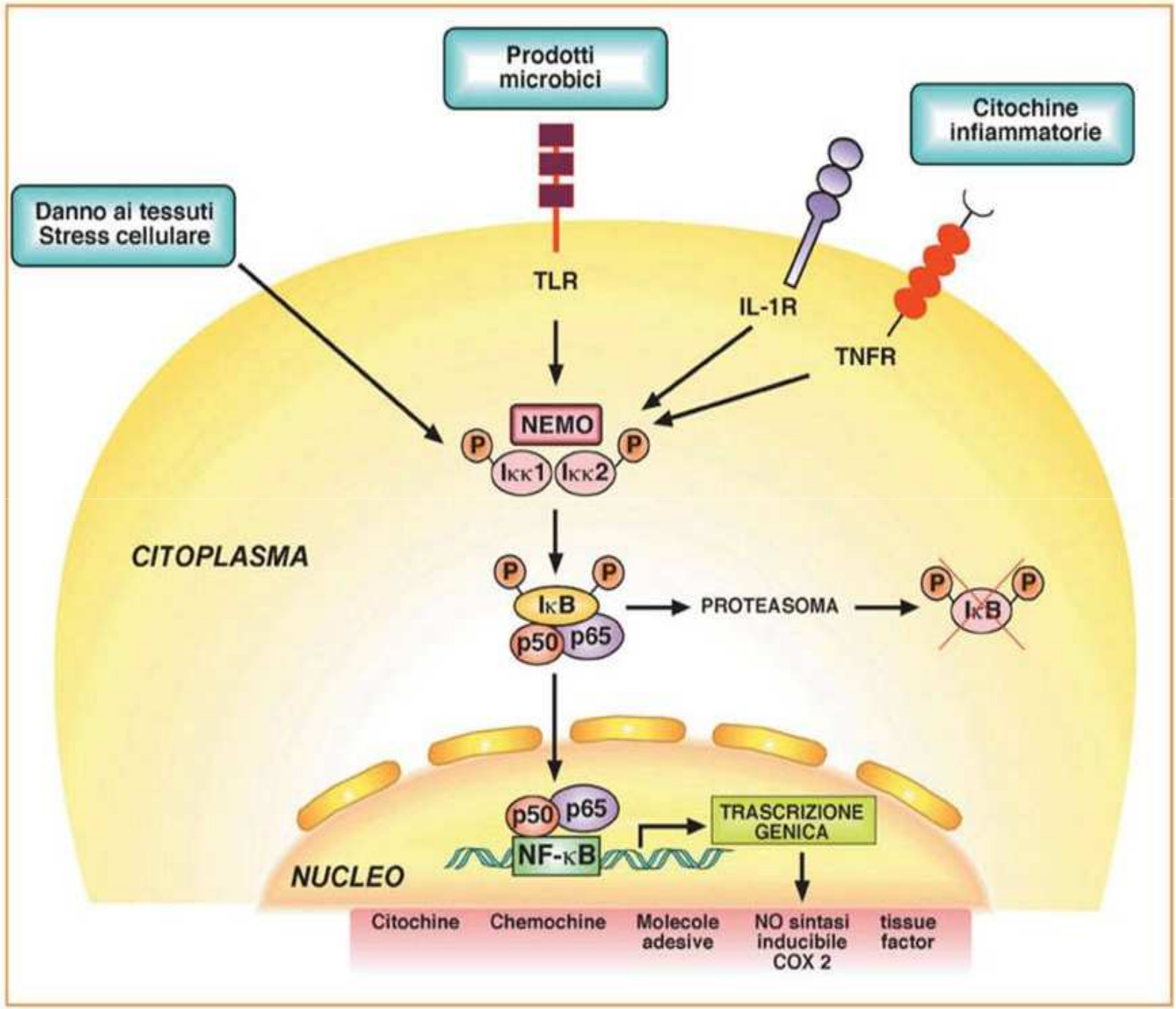
① Tissue injury; release of chemical signals (histamine, prostaglandins).

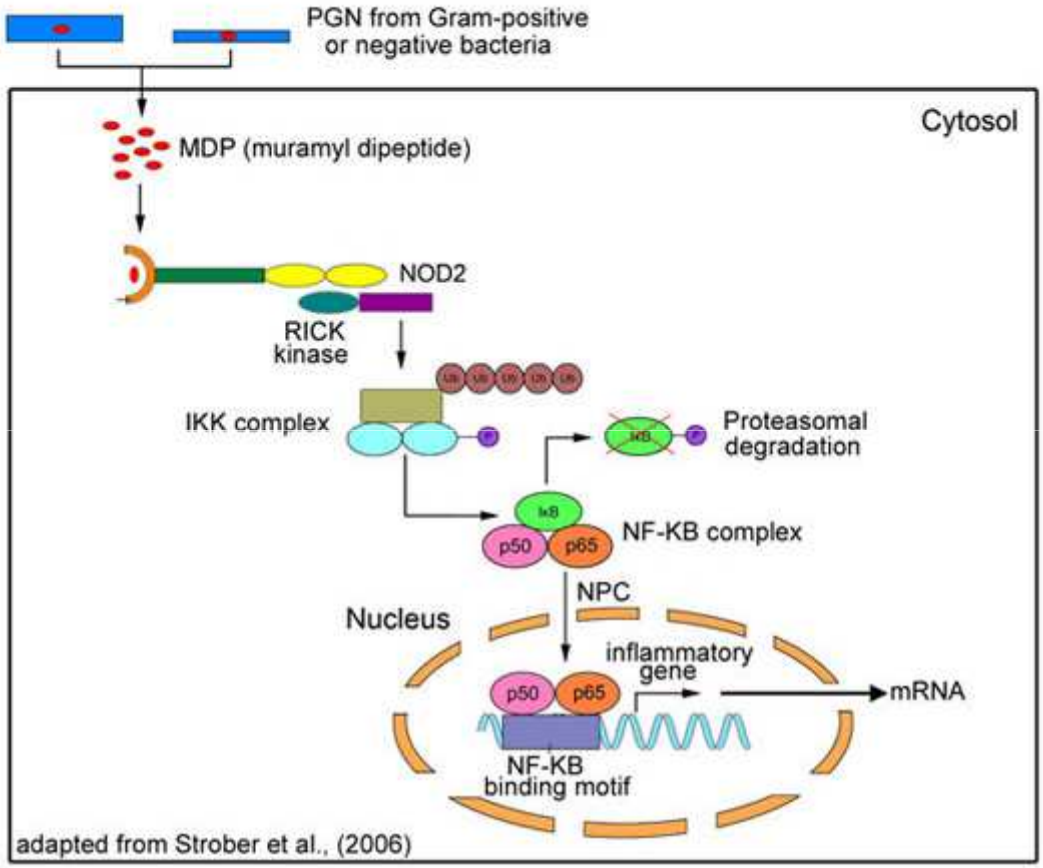
② Vasodilation (increased blood flow); increased vessel permeability; phagocyte migration.

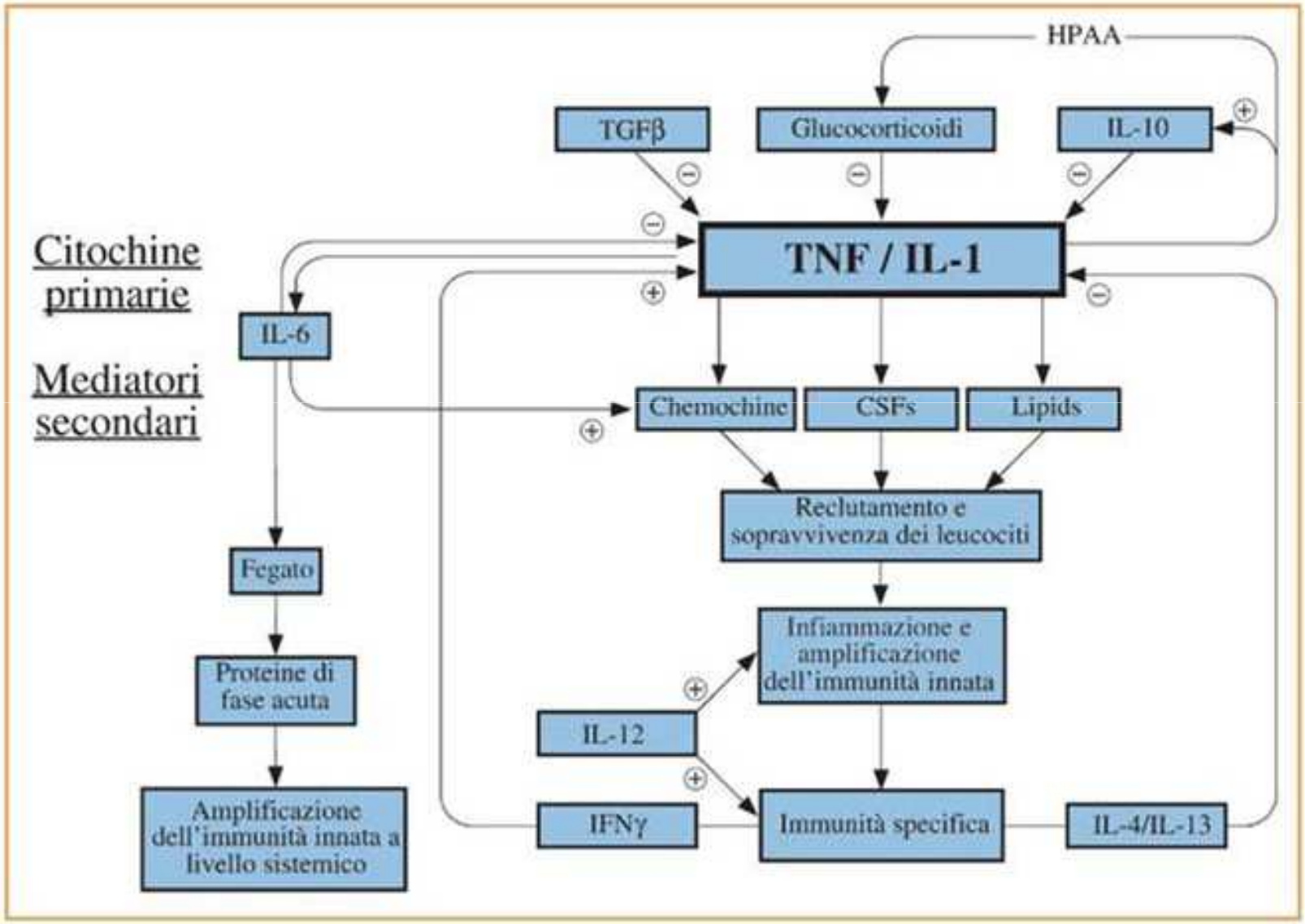
③ Phagocytes (macrophages and neutrophils) consume pathogens and cell debris; tissue heals.

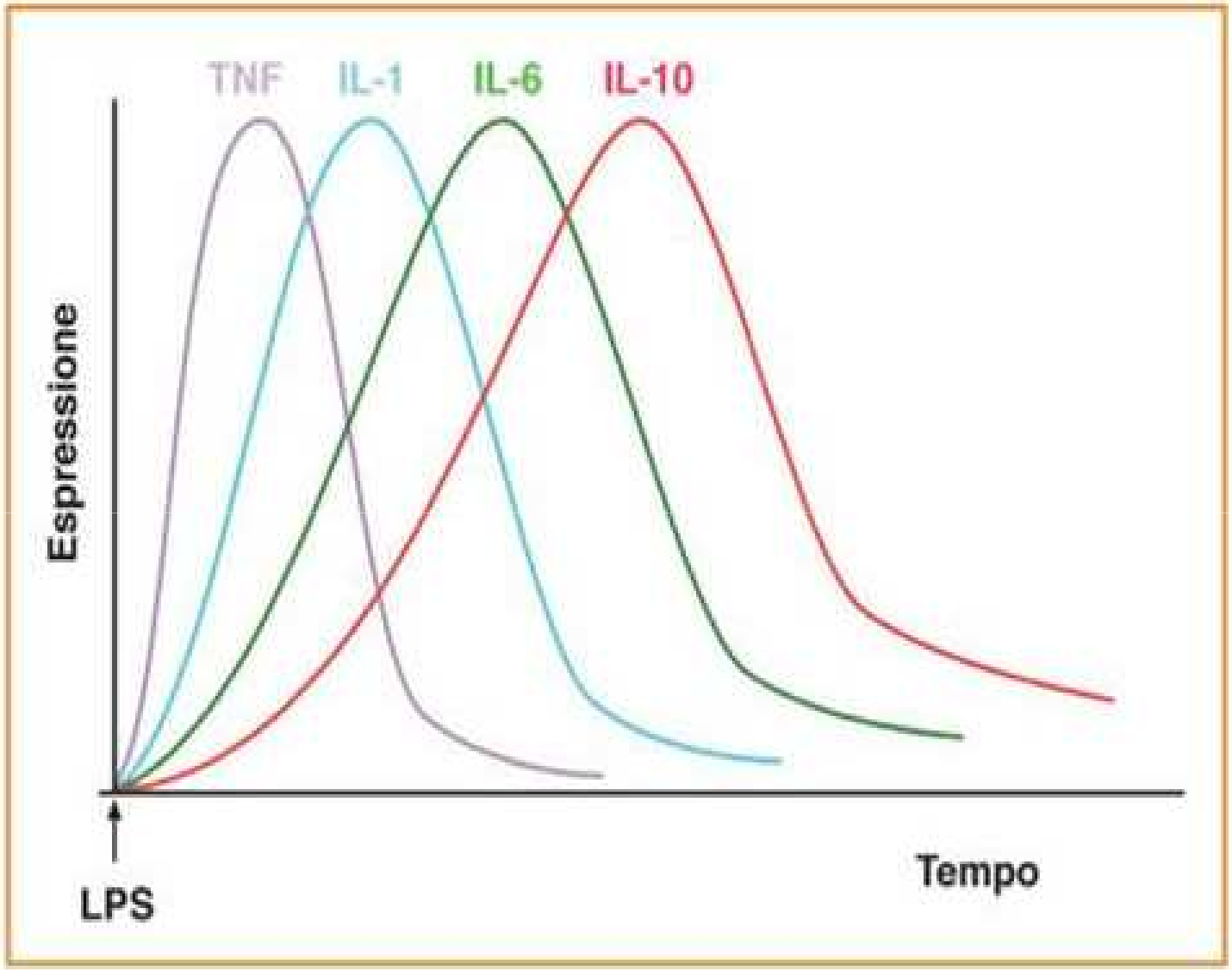

Inflammatory trigger
Infection
Tissue injury
Tissue stress and malfunction
Inflammation
Physiological purpose
Host defence against infection
Tissue-repair response
Adaption to stress, and restoration of a homeostatic state
Pathological consequences
Autoimmunity, inflammatory tissue damage and sepsis
Fibrosis, metaplasia and/or tumour growth
Shift in homeostatic set points, development of diseases of homeostasis and/or autoinflammatory diseases


Stranger model
Danger model
Pathogen
Necrotic cell death
PAMPs
Release of DAMPs
DC
PRR
DAMP receptor


Prodotti microbici
Citochine infiammatorie
Danno ai tessuti
Stress cellulare
TLR
IL-1R
TNFR
NEMO
P
IKK1
IKK2
CITOPLASMA
IκB
p50
p65
PROTEASOMA
TRASCRIZIONE GENICA
NF-κB
NUCLEO
Citochine
Chemochine
Molecole adesive
NO sintasi inducibile COX 2
tissue factor


PGN from Gram-positive
or negative bacteria
Cytosol
MDP (muramyl dipeptide)
NOD2
RICK
kinase
IKK complex
Proteasomal
degradation
IκB
p50
p65
NF-KB complex
NPC
Nucleus
inflammatory
gene
mRNA
NF-KB
binding motif
adapted from Strober et al., (2006)


HPAA
TGFβ
Glucocorticoidi
IL-10
Citochine primarie
TNF / IL-1
IL-6
Mediatori secondari
Chemochine
CSFs
Lipids
Reclutamento e sopravvivenza dei leucociti
Fegato
Infiammazione e amplificazione dell'immunità innata
Proteine di fase acuta
IL-12
Amplificazione dell'immunità innata a livello sistemico
IFNγ
Immunità specifica
IL-4/IL-13

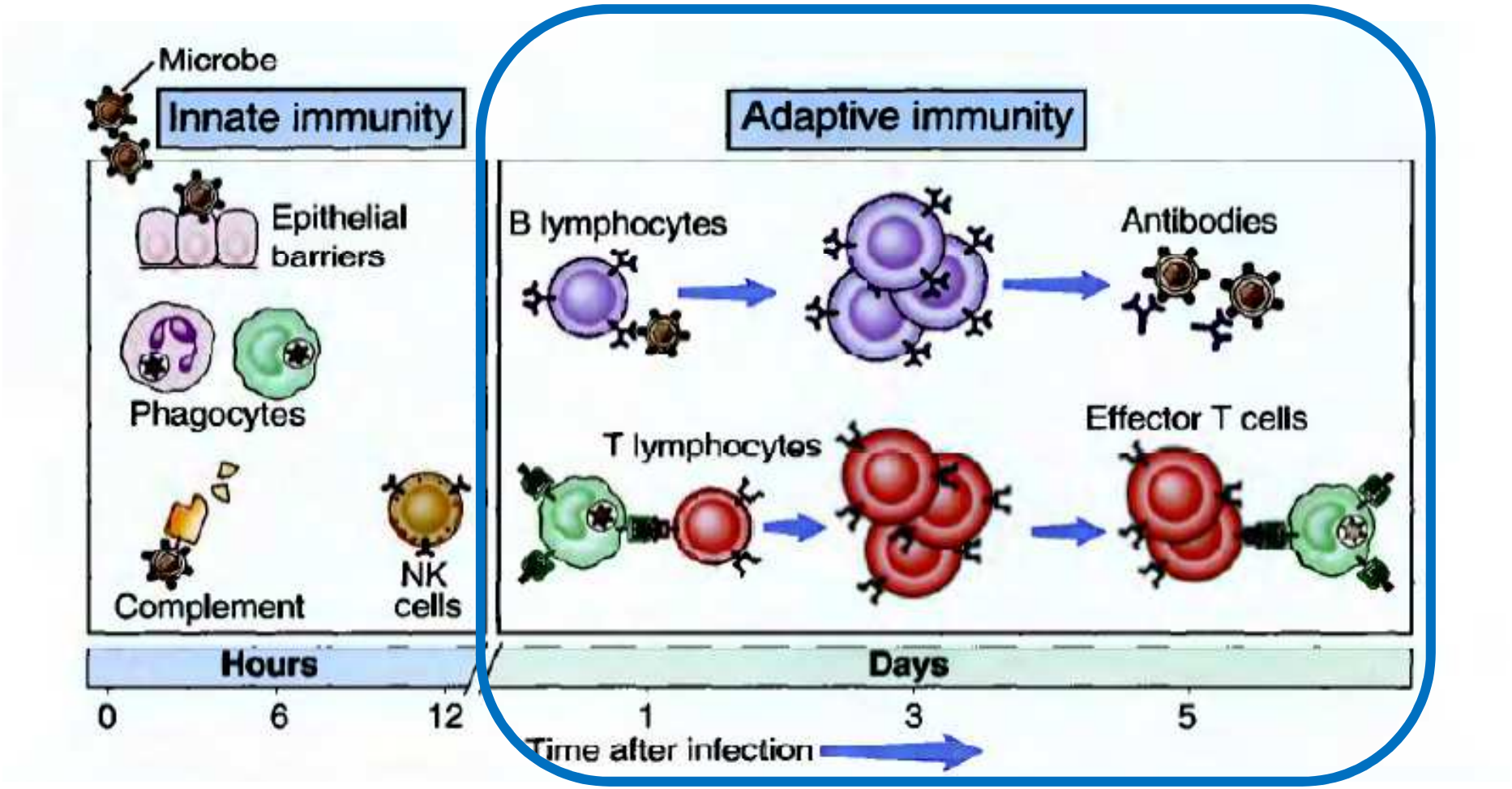

TNF
IL-1
IL-6
IL-10
Espressione
LPS
Tempo

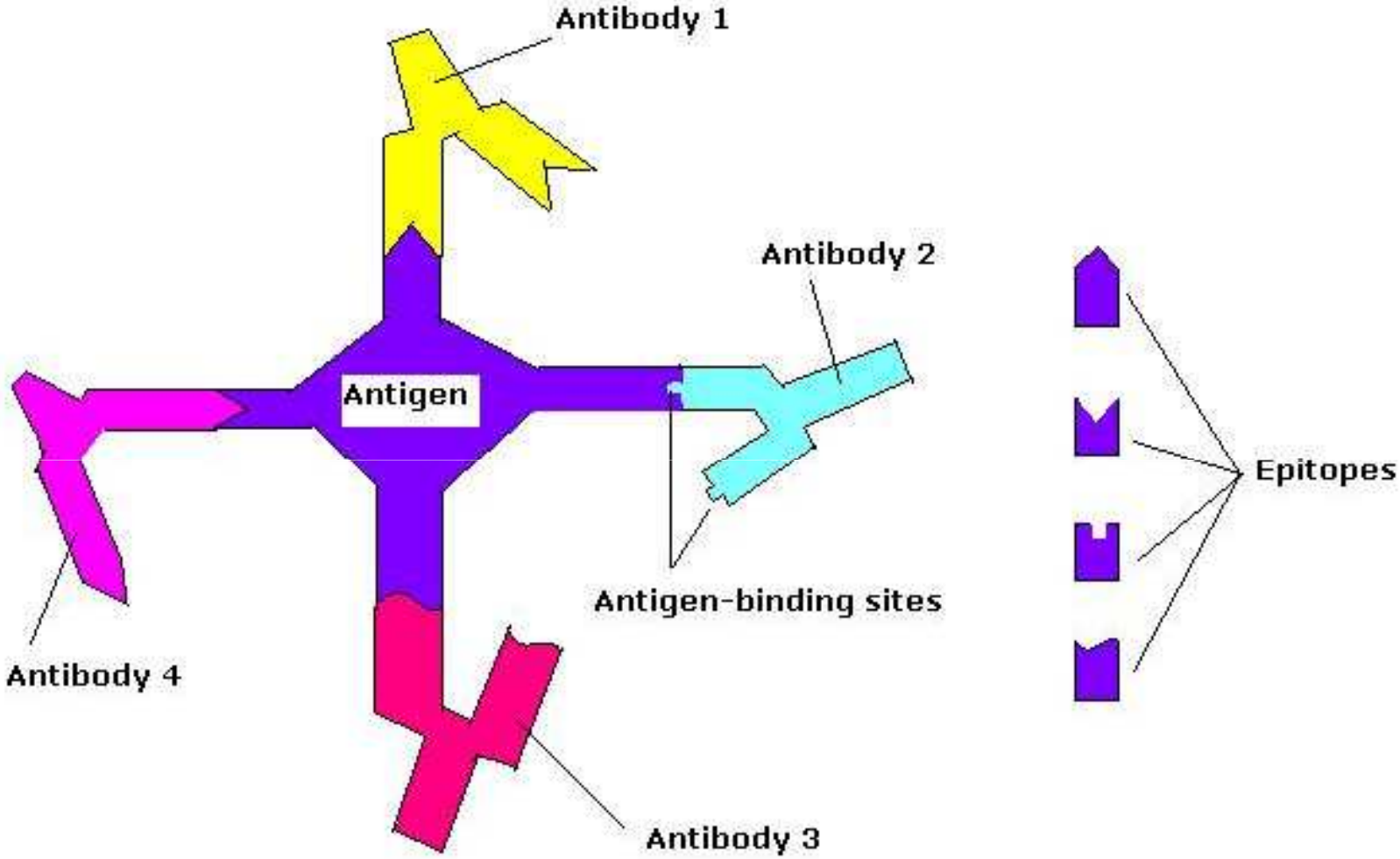
Immunità specifica (acquisita)

Barriere chimico-fisiche: sistema immune cutaneo, tessuto linfoide associato alle mucose e anticorpi prodotti con le secrezioni

Componenti cellulari: cellule linfocitarie (linfociti B e T), cellule presentanti l'antigene (APC), cellule ausiliarie ed endoteliali

Molecole circolanti: anticorpi prodotti dai linfociti B in risposta alla stimolazione con l'antigene e citochine prodotte da cellule linfoidi (IL-2, IL- 4,…)

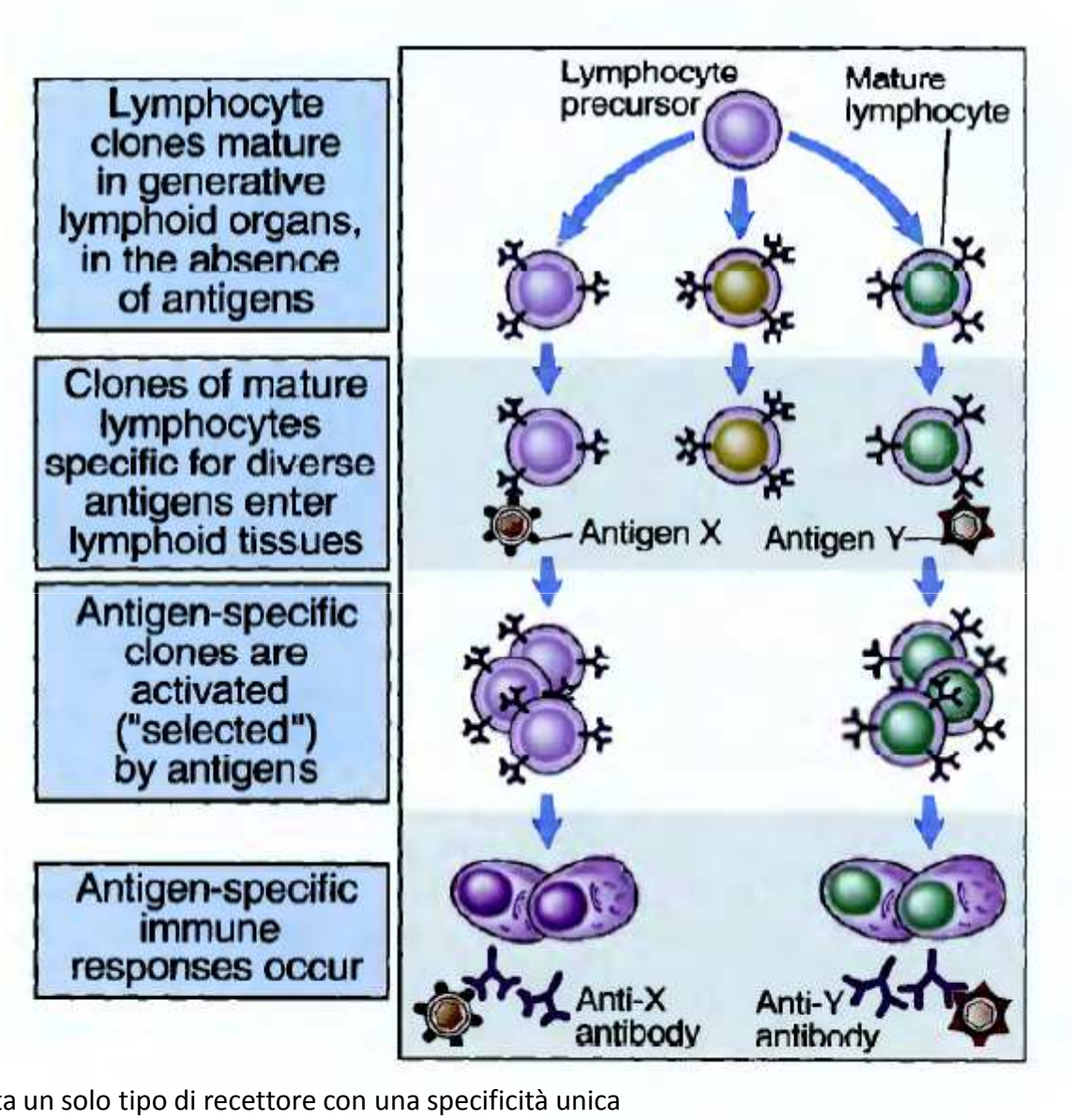



Antibody 1
Antibody 2
Antigen
Antigen-binding sites
Epitopes
Antibody 4
Antibody 3

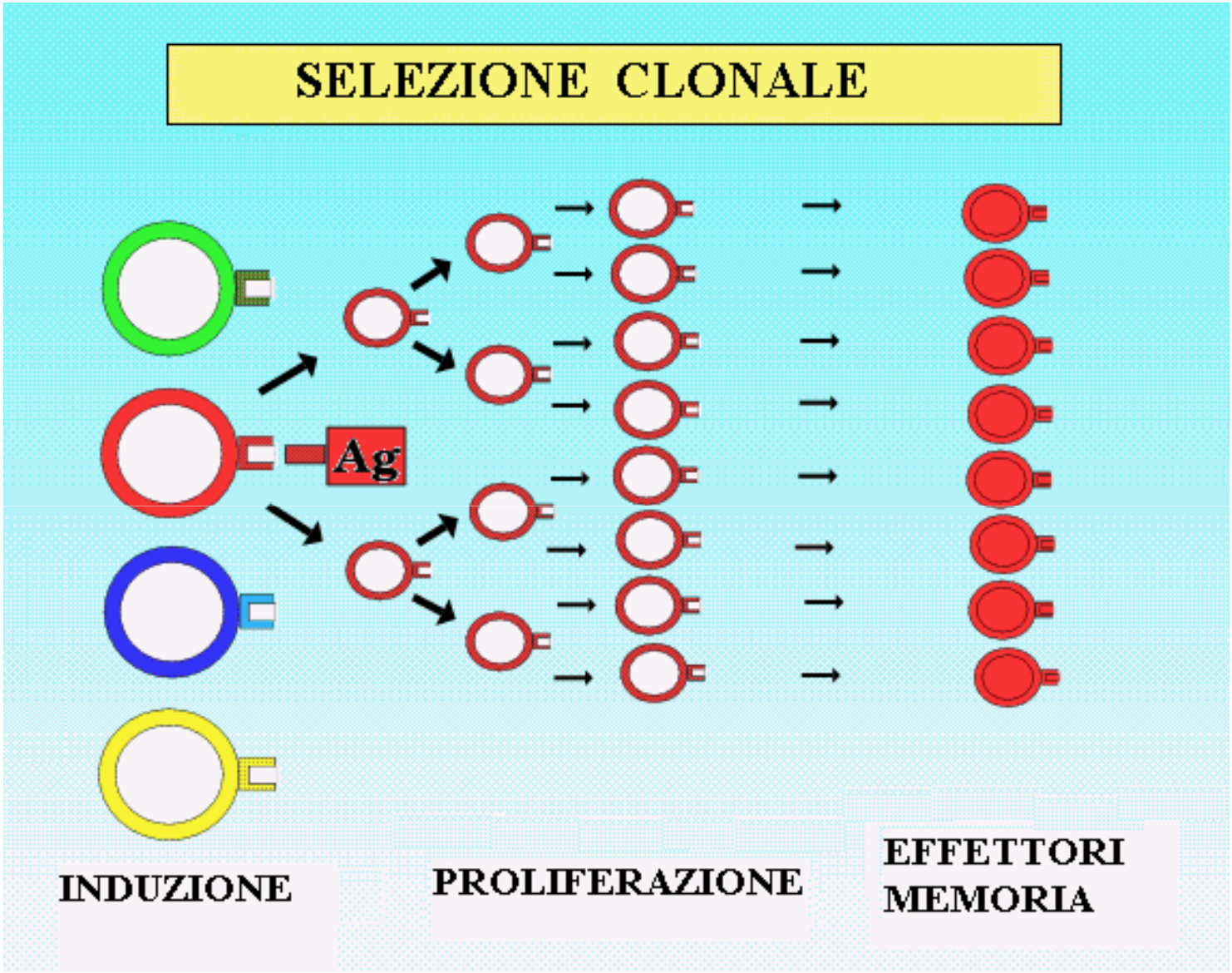
Specificità: per ogni antigene (generatore di anticorpi), o porzione di esso (determinante antigenico o epitopo), riconosciuto da recettori di membrana dei linfociti, si realizza una specifica risposta immune con la produzione di anticorpi (o una risposta cellulo-mediata specifica)

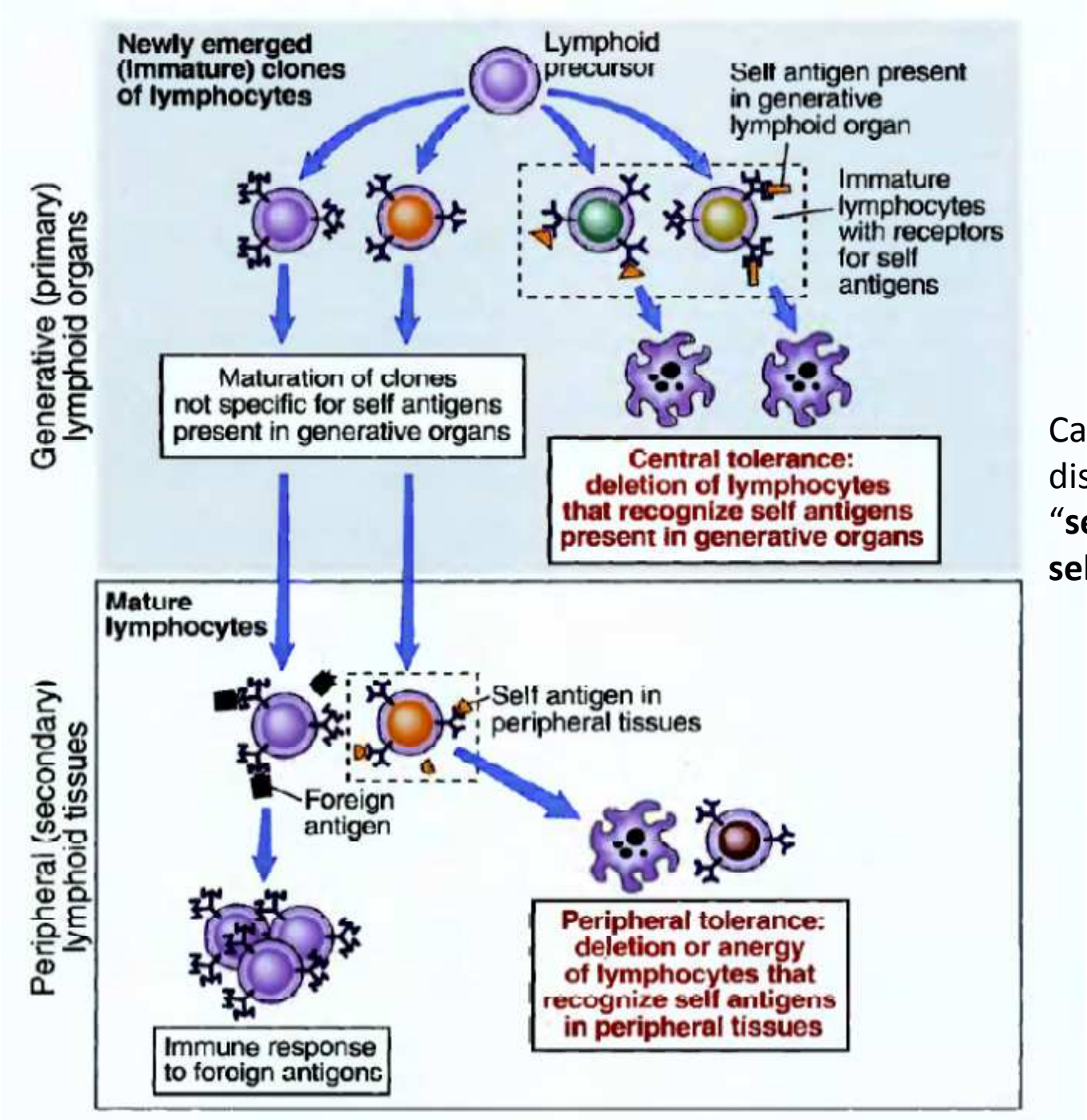
Diversità: gli antigeni sono in numero elevatissimo (10^9-10^{10}), il sistema immunitario riconosce e identifica tale numero di antigeni

Teoria della selezione clonale

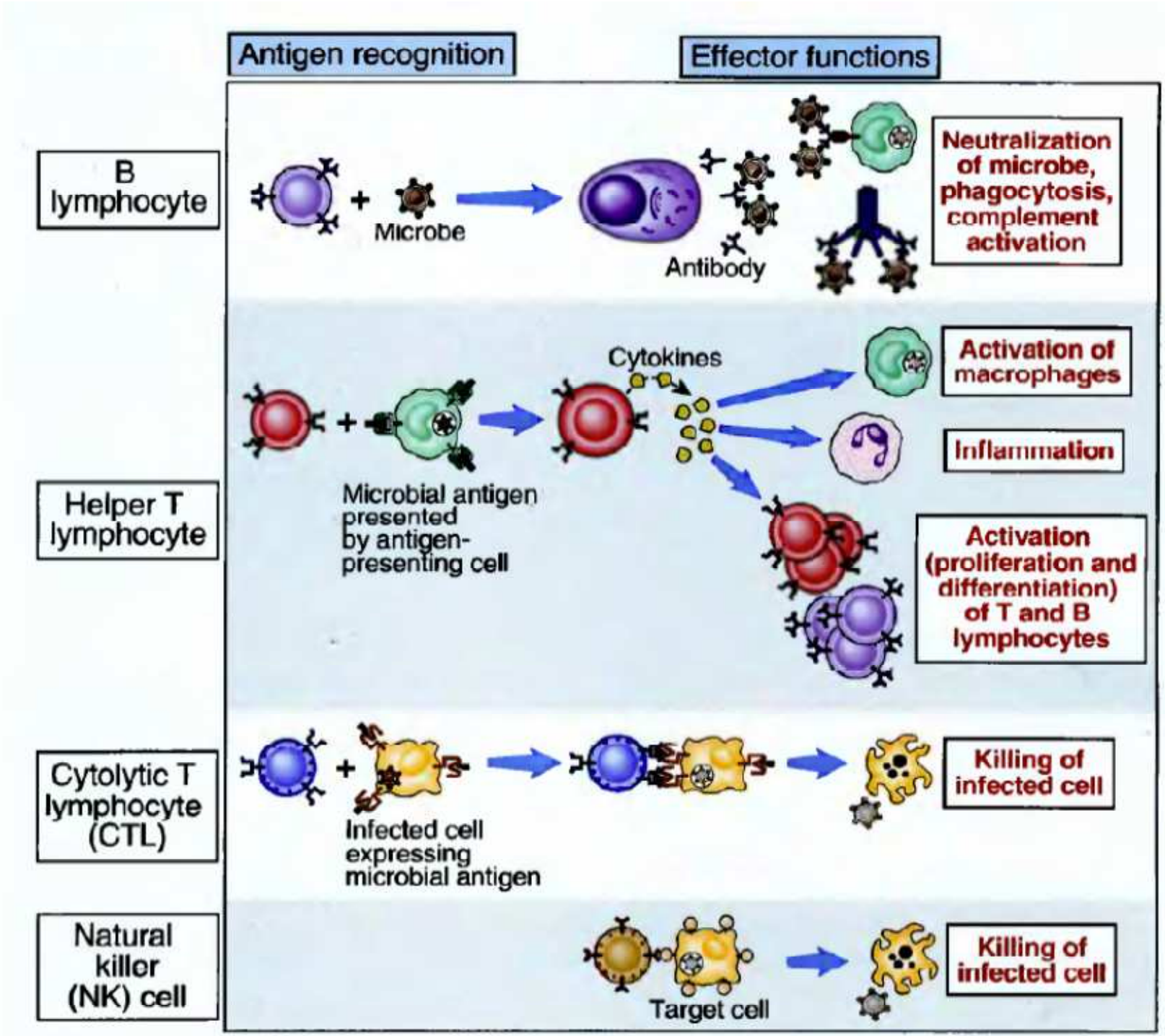


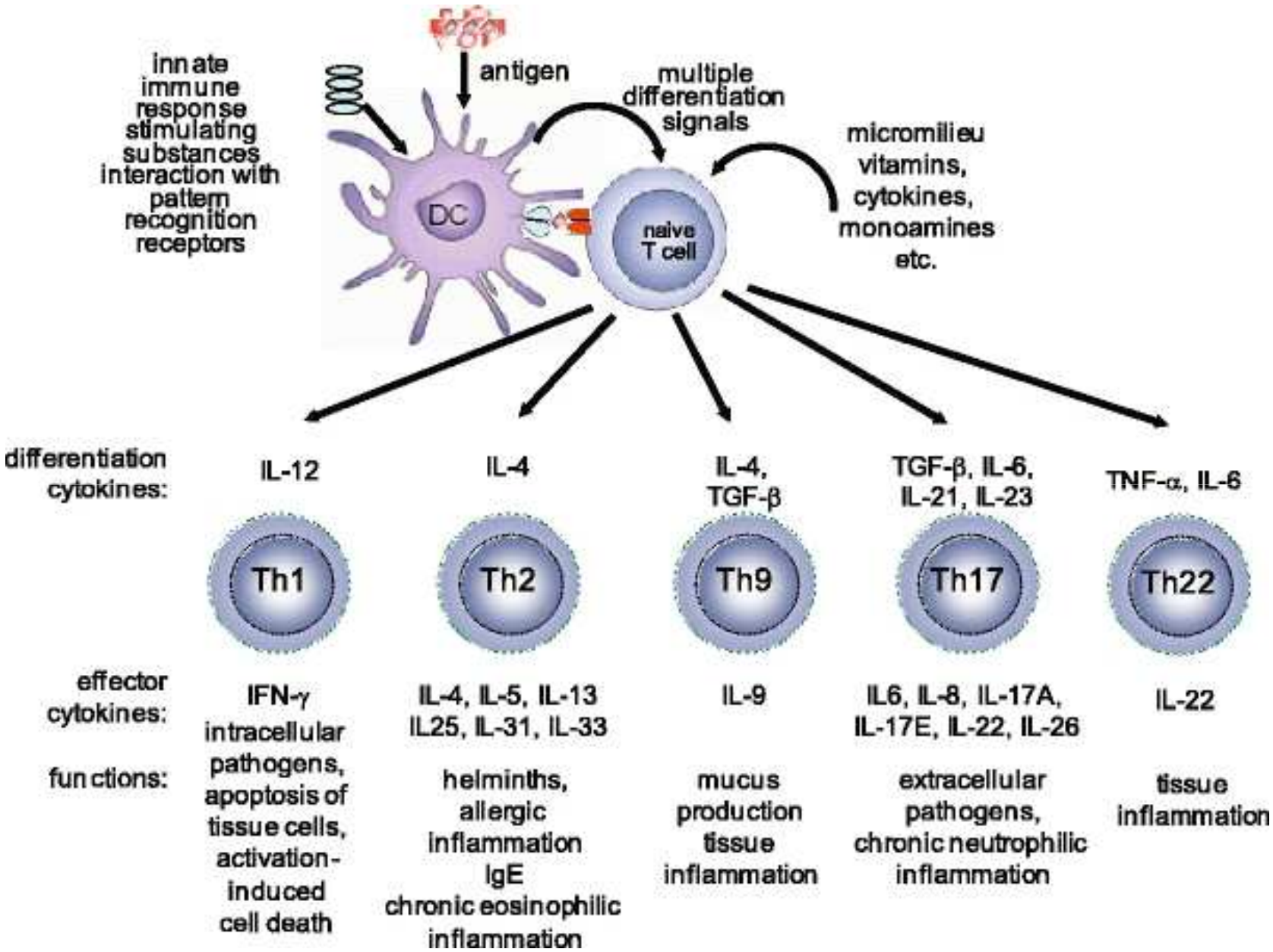
Ogni linfocita porta un solo tipo di recettore con una specificità unica
Per attivare la cellula, il recettore deve essere occupato
Le cellule derivate da un linfocita attivato avranno recettori dello stesso tipo della cellula originale
I linfociti che riconoscono i self vengono eliminati già all'inizio dello sviluppo

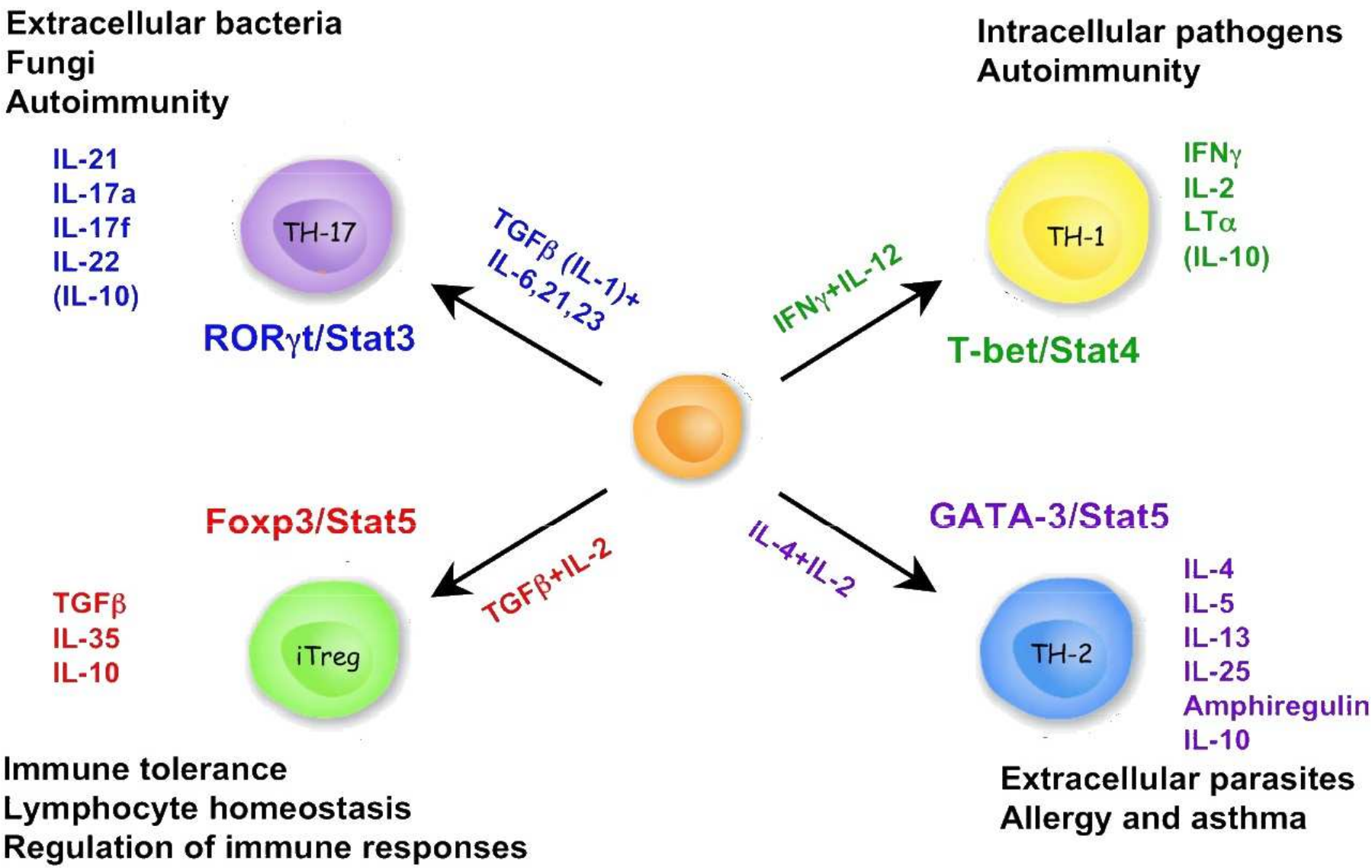

SELEZIONE CLONALE
Ag
INDUZIONE
PROLIFERAZIONE
EFFETTORI
MEMORIA



Capacità di discriminare il "**self**" dal "**non self**"


Antigen recognition
Effector functions
B lymphocyte
Microbe
Antibody
Neutralization of microbe, phagocytosis, complement activation
Helper T lymphocyte
Cytokines
Activation of macrophages
Inflammation
Microbial antigen presented by antigen-presenting cell
Activation (proliferation and differentiation) of T and B lymphocytes
Cytolytic T lymphocyte (CTL)
Killing of infected cell
Infected cell expressing microbial antigen
Natural killer (NK) cell
Killing of infected cell
Target cell


innate immune response stimulating substances interaction with pattern recognition receptors
antigen
multiple differentiation signals
micromilieu vitamins, cytokines, monoamines etc.
DC
naive T cell
differentiation cytokines:
IL-12
IL-4
IL-4, TGF-β
TGF-β, IL-6, IL-21, IL-23
TNF-α, IL-6
Th1
Th2
Th9
Th17
Th22
effector cytokines:
IFN-γ
IL-4, IL-5, IL-13 IL25, IL-31, IL-33
IL-9
IL6, IL-8, IL-17A, IL-17E, IL-22, IL-26
IL-22
functions:
intracellular pathogens, apoptosis of tissue cells, activation-induced cell death
helminths, allergic inflammation IgE chronic eosinophilic inflammation
mucus production tissue inflammation
extracellular pathogens, chronic neutrophilic inflammation
tissue inflammation


Extracellular bacteria
Fungi
Autoimmunity
Intracellular pathogens
Autoimmunity
IL-21
IL-17a
IL-17f
IL-22
(IL-10)
TH-17
RORγt/Stat3
TGFβ (IL-1)+
IL-6,21,23
IFNγ+IL-12
TH-1
IFNγ
IL-2
LTα
(IL-10)
T-bet/Stat4
Foxp3/Stat5
TGFβ
IL-35
IL-10
iTreg
TGFβ+IL-2
IL-4+IL-2
GATA-3/Stat5
TH-2
IL-4
IL-5
IL-13
IL-25
Amphiregulin
IL-10
Immune tolerance
Lymphocyte homeostasis
Regulation of immune responses
Extracellular parasites
Allergy and asthma

Genetics

More recently, the search for IBD-associated genes has used genome-wide association studies (GWAS) that assess single-nucleotide polymorphisms. The number of genes identified by GWAS is increasing rapidly (already numbering more than 30), but along with **NOD2**, two Crohn disease–related genes of particular interest are **ATG16L1 (autophagy-related 16-like)**, a part of the autophagosome pathway that is critical to host cell responses to intracellular bacteria and, perhaps, epithelial homeostasis, and **IRGM (immunity-related GTPase M)**, which is also involved in autophagy and clearance of intracellular bacteria. NOD2, ATG16L1, and IRGM are expressed in multiple cell types, and their precise roles in Crohn disease pathogenesis have yet to be defined. However, like NOD2, ATG16L1 and IRGM are related to recognition and response to intracellular pathogens, supporting the hypothesis that inappropriate immune reactions to luminal bacteria are an important component of IBD pathogenesis. None of these genes are associated with ulcerative colitis. However, some polymorphisms of the IL-23 receptor are protective in both Crohn disease and ulcerative colitis.

Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci

We undertook a meta-analysis of six Crohn's disease genome-wide association studies (GWAS) comprising 6,333 affected individuals (cases) and 15,056 controls and followed up the top association signals in 15,694 cases, 14,026 controls and 414 parent-offspring trios. We identified 30 new susceptibility loci meeting genome-wide significance ($P < 5 \times 10^{-8}$). A series of *in silico* analyses highlighted particular genes within these loci and, together with manual curation, implicated functionally interesting candidate genes including *SMAD3*, *ERAP2*, *IL10*, *IL2RA*, *TYK2*, *FUT2*, *DNMT3A*, *DENND1B*, *BACH2* and *TAGAP*. Combined with previously confirmed loci, these results identify 71 distinct loci with genome-wide significant evidence for association with Crohn's disease.

Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47

Genome-wide association studies and candidate gene studies in ulcerative colitis have identified 18 susceptibility loci. We conducted a meta-analysis of six ulcerative colitis genome-wide association study datasets, comprising 6,687 cases and 19,718 controls, and followed up the top association signals in 9,628 cases and 12,917 controls. We identified 29 additional risk loci ($P < 5 \times 10^{-8}$), increasing the number of ulcerative colitis–associated loci to 47. After annotating associated regions using GRAIL, expression quantitative trait loci data and correlations with non-synonymous SNPs, we identified many candidate genes that provide potentially important insights into disease pathogenesis, including *IL1R2*, *IL8RA-IL8RB*, *IL7R*, *IL12B*, *DAP*, *PRDM1*, *JAK2*, *IRF5*, *GNA12* and *LSP1*. The total number of confirmed inflammatory bowel disease risk loci is now 99, including a minimum of 28 shared association signals between Crohn's disease and ulcerative colitis.

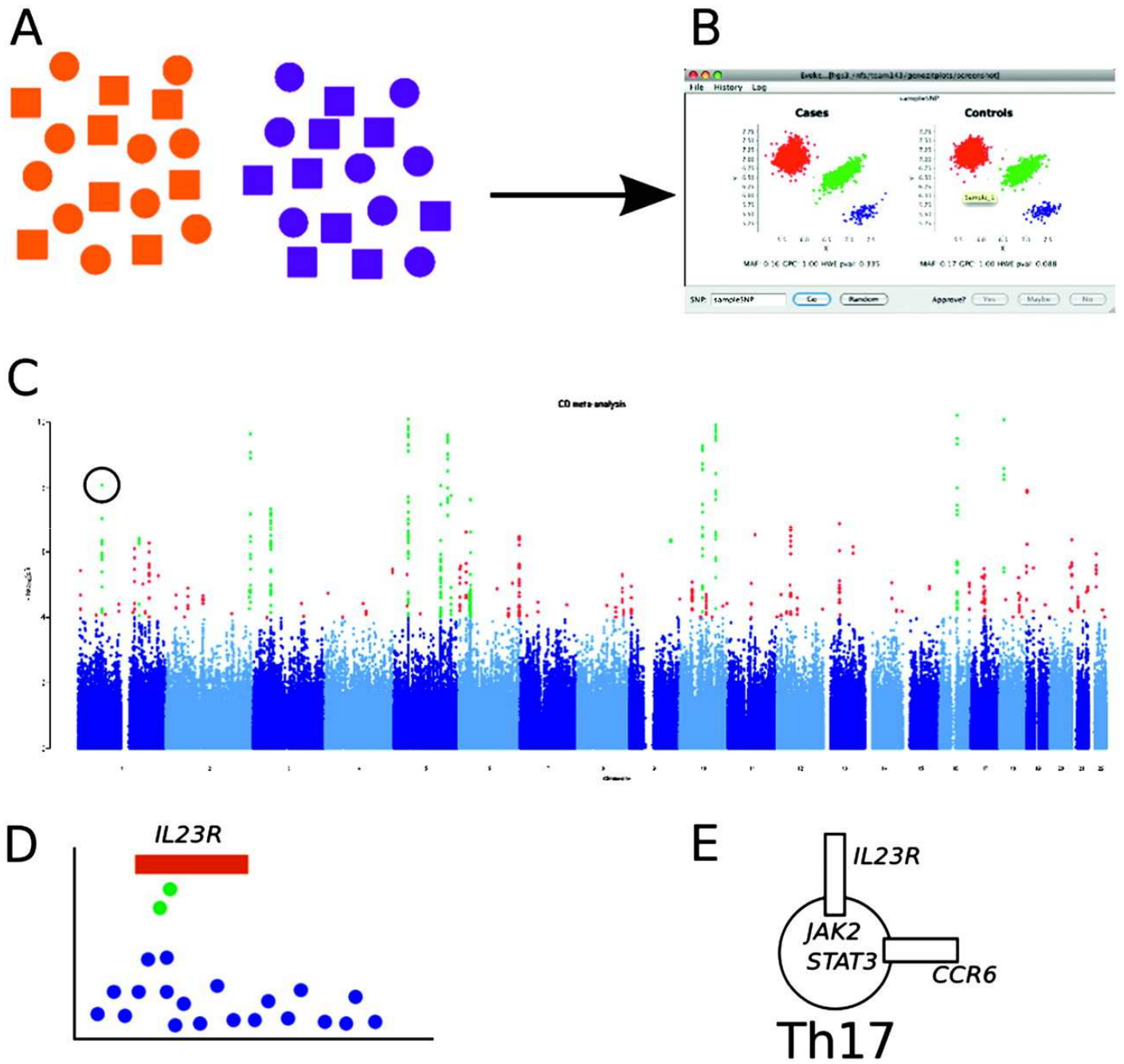
Ulcerative colitis and Crohn's disease represent the two major forms of inflammatory bowel disease (IBD, MIM#266600), which together affect approximately 1 in 250 people in Europe, North America and Australasia. Clinical features, epidemiological data and genetic evidence suggest that ulcerative colitis and Crohn's disease are related polygenic diseases. In contrast to Crohn's disease, bowel inflammation in ulcerative colitis is limited to the colonic mucosa. Although disease-related mortality is low, morbidity remains high, and 10%–20% of affected individuals will undergo colectomy. Though the precise etiology is unknown, the current hypothesis is a dysregulated mucosal immune response to commensal gut flora in genetically susceptible individuals[1]. Recent genome-wide and candidate gene association studies have identified 18 susceptibility loci for ulcerative colitis, including seven that overlap with Crohn's disease (for example, *IL23* pathway genes, *NKX2-3* and *IL10*). Established risk loci specific for ulcerative colitis (*HNF4A*, *CDH1* and *LAMB1*) have highlighted the role of defective barrier function in disease pathogenesis[2].

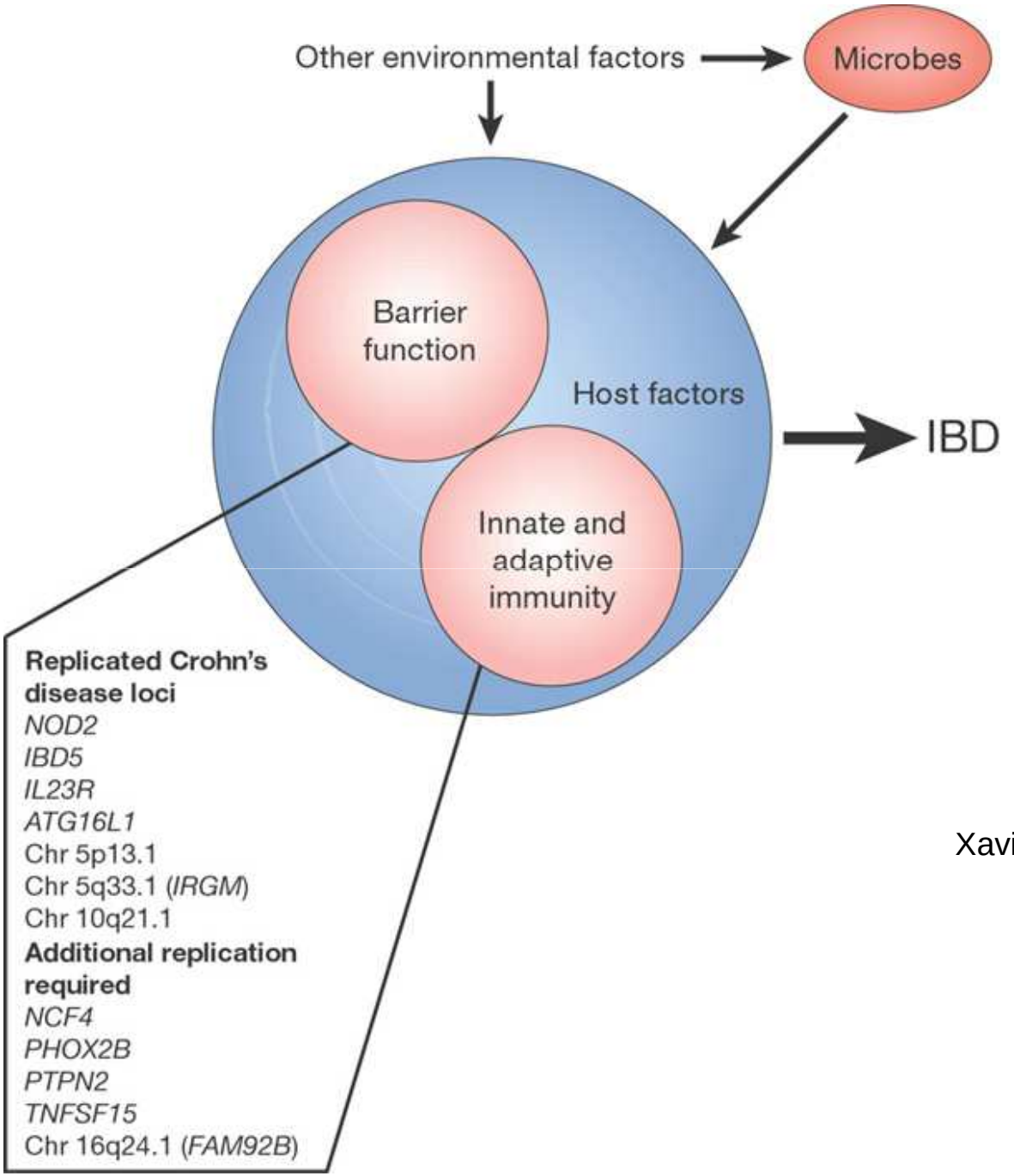
What is a GWAS?

- A genome-wide association study is an approach that involves rapidly scanning **markers across genome** (≈0.5M or 1M) of **many people** (≈2K) to find genetic variations associated with a particular disease

- A large number of subjects are needed because
  (1) associations between SNPs and causal variants are expected to show **low odds ratios**, typically below 1.5
  (2) In order to obtain a reliable signal, given the very large number of tests that are required, associations must show a high level of significance to survive the multiple testing correction

- Such studies are particularly useful in finding genetic variations that contribute to common, complex diseases


A
B
Cases
Controls
C
CD meta-analysis
D
IL23R
E
IL23R
JAK2
STAT3
CCR6
Th17

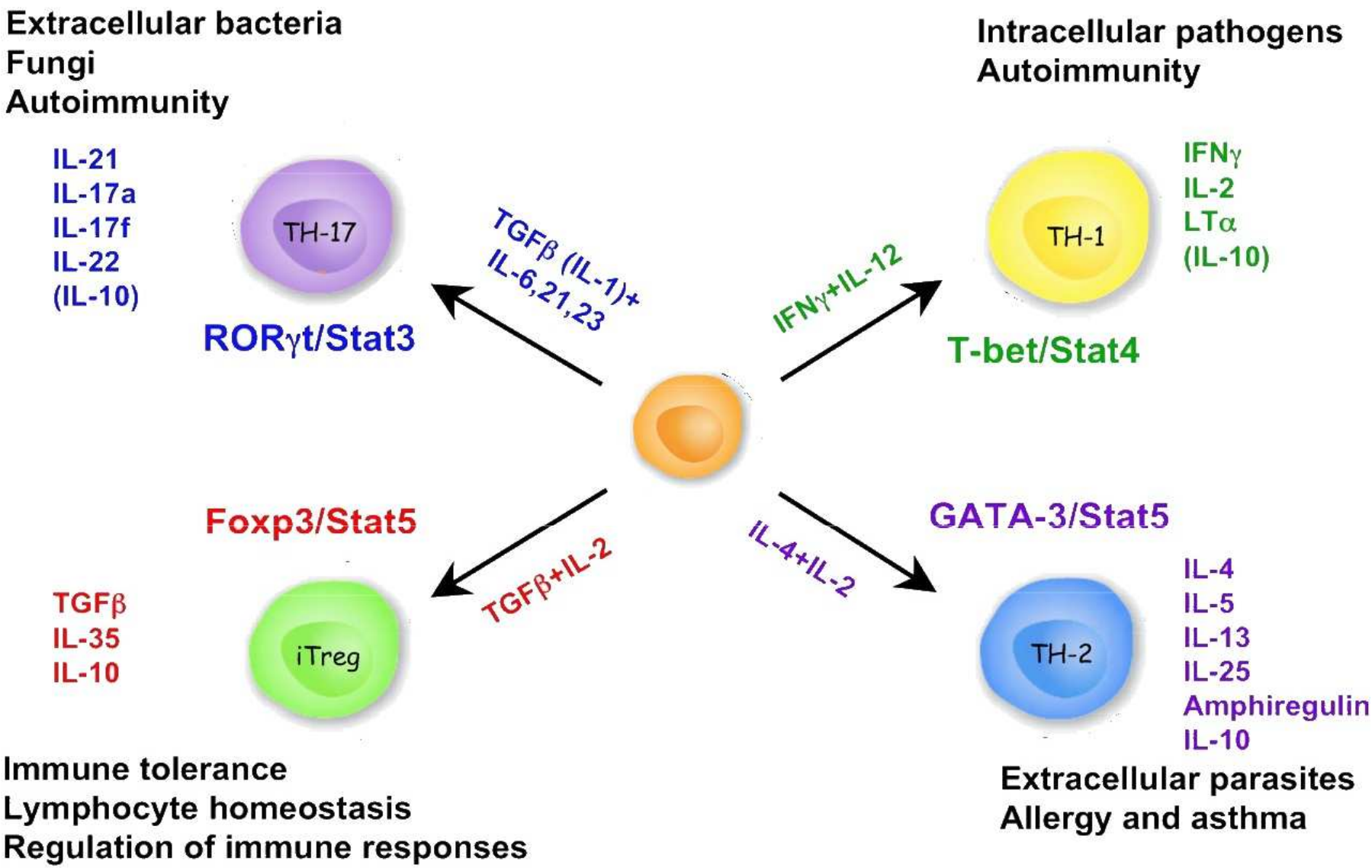

Other environmental factors
Microbes
Barrier function
Host factors
Innate and adaptive immunity
IBD
Replicated Crohn's disease loci
NOD2
IBD5
IL23R
ATG16L1
Chr 5p13.1
Chr 5q33.1 (IRGM)
Chr 10q21.1
Additional replication required
NCF4
PHOX2B
PTPN2
TNFSF15
Chr 16q24.1 (FAM92B)

Xavier 2007

Mucosal immune responses

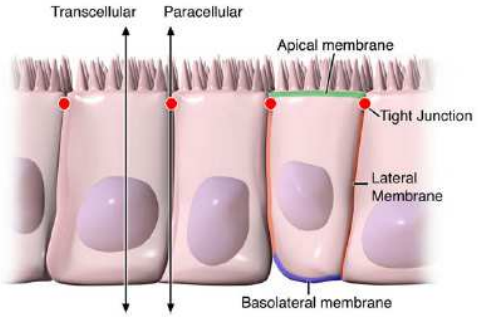
Although the mechanisms by which **mucosal immunity** contributes to ulcerative colitis and Crohn disease pathogenesis are still being deciphered, **immunosuppression** remains the mainstay of IBD **therapy**. Polarization of helper T cells to the TH1 type is well-recognized in Crohn disease, and emerging data suggest that TH17 T cells also contribute to disease pathogenesis. Consistent with this, certain polymorphisms of the **IL-23 receptor** confer protection from Crohn disease and ulcerative colitis. IL-23 is involved in the development and maintenance of TH17 cells, suggesting that the protective IL-23 receptor polymorphisms may attenuate pro-inflammatory TH17 responses in Crohn disease and ulcerative colitis.

Some data suggest that ulcerative colitis is a TH2-mediated disease, and this is consistent with observations of increased mucosal IL-13 in ulcerative colitis patients. However, the protection afforded by IL-23 receptor polymorphisms and effectiveness of **anti-TNF therapy** in some ulcerative colitis patients seems to support roles for TH1 and TH17 cells. A recent report linking polymorphisms near the **IL-10 gene** to ulcerative colitis, but not Crohn disease, further emphasizes the importance of **immunoregulatory signals** in IBD pathogenesis. Overall it is likely that some combination of derangements that activate mucosal immunity and suppress immunoregulation contribute to the development of ulcerative colitis and Crohn disease. The relative roles of innate and adaptive arms of the immune system are presently the subject of intense scrutiny.


Extracellular bacteria
Fungi
Autoimmunity
Intracellular pathogens
Autoimmunity
IL-21
IL-17a
IL-17f
IL-22
(IL-10)
TH-17
RORγt/Stat3
TGFβ (IL-1)+
IL-6,21,23
IFNγ+IL-12
TH-1
IFNγ
IL-2
LTα
(IL-10)
T-bet/Stat4
Foxp3/Stat5
TGFβ
IL-35
IL-10
iTreg
TGFβ+IL-2
IL-4+IL-2
GATA-3/Stat5
TH-2
IL-4
IL-5
IL-13
IL-25
Amphiregulin
IL-10
Immune tolerance
Lymphocyte homeostasis
Regulation of immune responses
Extracellular parasites
Allergy and asthma

Epithelial defects

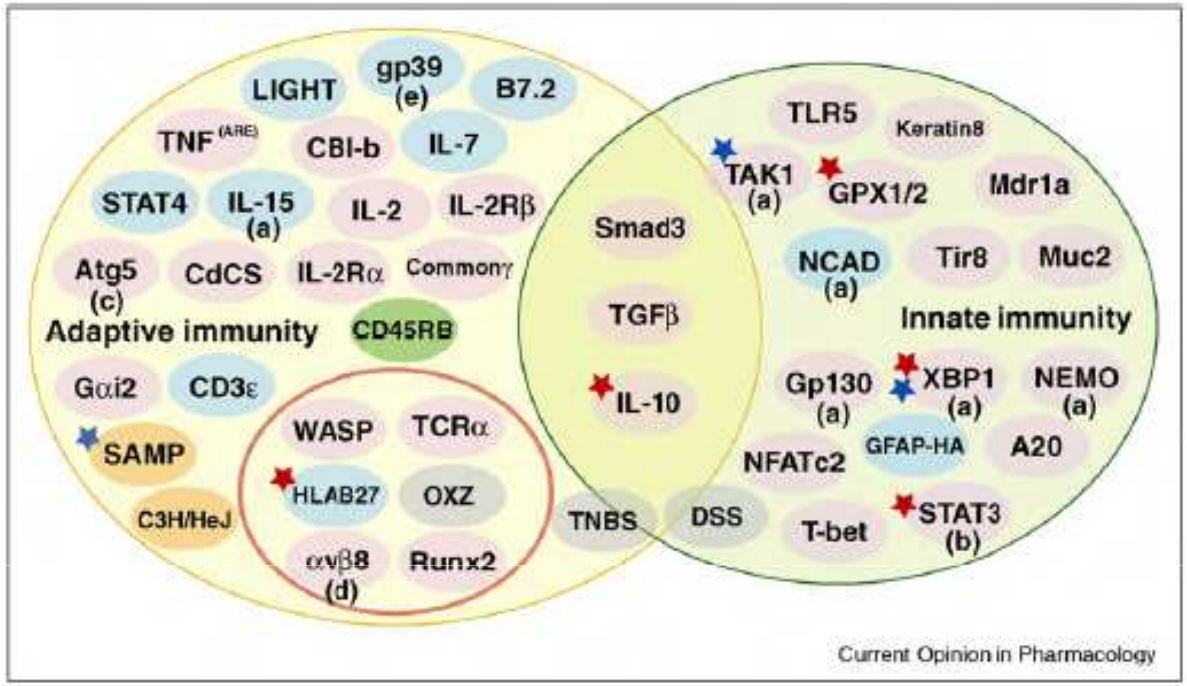
A variety of epithelial defects have been described in both Crohn disease and ulcerative colitis. For example, defects in **intestinal epithelial tight junction barrier** function are present in Crohn disease patients and a subset of their healthy first-degree relatives. In patients with Crohn disease and their relatives, this barrier dysfunction is associated with *NOD2* polymorphisms, and experimental models demonstrate that **barrier dysfunction can activate innate and adaptive mucosal immunity and sensitize subjects to disease**. Moreover, mutation of the organic cation transporter *SLC22A4* in Crohn disease suggests that defective transepithelial transport may also be related to IBD pathogenesis. Defects in the extracellular barrier formed by secreted mucin may also contribute. Interestingly, polymorphisms in ***ECM1*** (extracellular matrix protein 1), which inhibits matrix metalloproteinase 9, are associated with ulcerative colitis but not Crohn disease. While the pathogenic relevance of *ECM1* mutations is not understood, it is notable that **inhibition of matrix metalloproteinase 9 reduces the severity of colitis in experimental models**. Finally, the **Paneth cell** granules, which contain antibacterial peptides termed defensins, are abnormal in Crohn disease patients carrying ***ATG16L1*** mutations, suggesting that defective epithelial anti-microbial function contributes to IBD. Thus, while the details are incompletely defined and probably differ between Crohn disease and ulcerative colitis, deranged epithelial function is a critical component of IBD pathogenesis.



Microbiota

The abundance of microbiota in the GI lumen is overwhelming, amounting to as much as 10^{12} organisms per milliliter in the colon and 50% of fecal mass. In total, these organisms greatly outnumber human cells in our bodies, meaning that, at a cellular level, **we are only about 10% human**. Although the composition of this dense microbial population tends to be stable within individuals over at least several years, it can be modified by diet and there is significant variation between individuals. In addition to the luminal microbiota, the more limited microbial population that inhabits the intestinal mucous layer may have the greatest impact on health. Despite growing evidence that intestinal microbiota contribute to IBD pathogenesis, their precise role remains to be defined and is probably different in ulcerative colitis and Crohn disease. For example, **antibodies against the bacterial protein flagellin are associated with *NOD2* polymorphisms** as well as stricture formation, perforation, and small-bowel involvement in patients with Crohn disease, but are uncommon in ulcerative colitis patients. In addition, some **antibiotics**, e.g. metronidazole, can be helpful in management of Crohn disease, and broad-spectrum antibiotics can prevent disease in some experimental models of IBD. Ongoing studies suggest that ill-defined mixtures containing **probiotic, or beneficial, bacteria may combat disease** in experimental models as well as some IBD patients, although the mechanisms responsible are not well understood.

Mizoguchi e Mizoguchi , Current Opinion in Pharmacology 2010, 10:578–587



Animal models of IBD. Many animal models of IBD are currently available to use, including knockout mice (pink), transgenic mice (blue), congenic mice (orange), chemically induced models (gray), and cell-transfer model (green). There are genetically engineered mice that have specific deletion of a target molecule in epithelial cells **(a)**, epithelial cells/macrophages **(b)**, thymic epithelial cells **(c)**, dendritic cells **(d)**, or B cells **(e)**. Mouse models, which have been used for studying UC, are surrounded by an orange circle. Mouse models, which develop ileitis spontaneously, are indicated by blue stars. Mouse models, which lack an IBD-associated gene, are highlighted by red stars. The abbreviations used in this figure are: A20, also known as TNF-induced protein 3; Atg, autophagy gene; αvβ8, integrin αvβ8; CBl-b, E3 ubiquitin ligase; DSS, dextran sulfate sodium; Gαi2, G protein αi2; GFAP-Hatg, transgenic mice in which entero glia is specifically disrupted; GPX, glutathione peroxidase; HLA/B27, HLAB27/human β2 microglobulin transgenic rats; LIGHT, a TNF superfamily member; Mdr, multiple drug resistance; Muc, mucin; NCAD, transgenic mice which overexpress dominant negative N-cadherin in the intestinal epithelial cells; NEMO, NFκB essential modulator; NFAT, nuclear factor of activated T cells; SAMP, a congenic mouse developing ileitis; STAT, signal transducer and activator of transcription; TAK, TGFβ-activated kinase; T-bet, T-box transcription factor; TCR, T cell receptor; Tir8, also known as SIGIRR (single Ig IL-1-realted receptor); TLR, toll-like receptor; TNBS, trinitrobenzene sulfonic acid; $TNF^{\Delta ARE}$, mice lacking TNF AU-rich elements; WASP, Wiskott–Aldrich syndrome protein; XBP1, X-box binding protein.

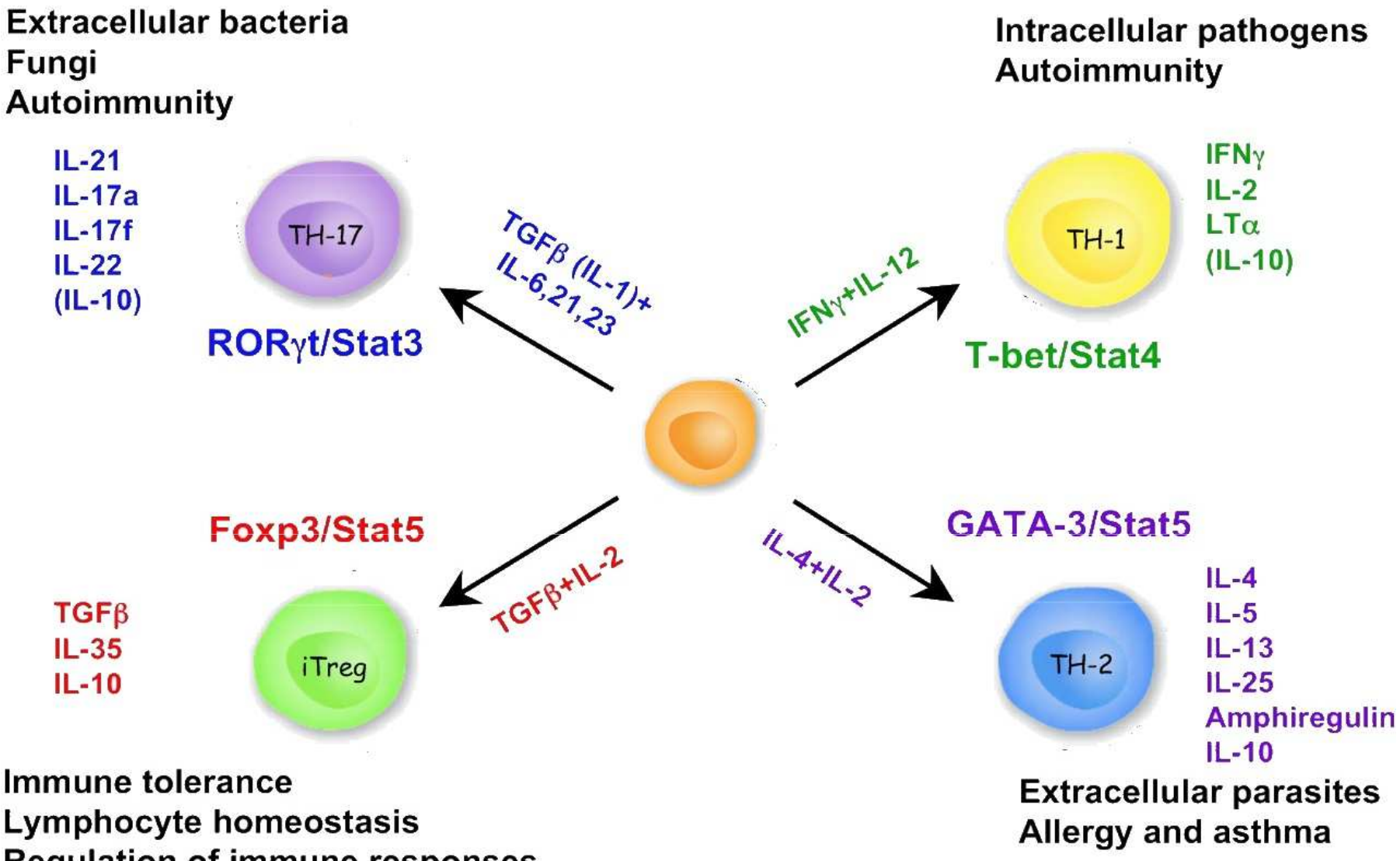
Saleh e Elson, Immunity 34, March 25, 2011

Table 1. Experimental Models of Colitis

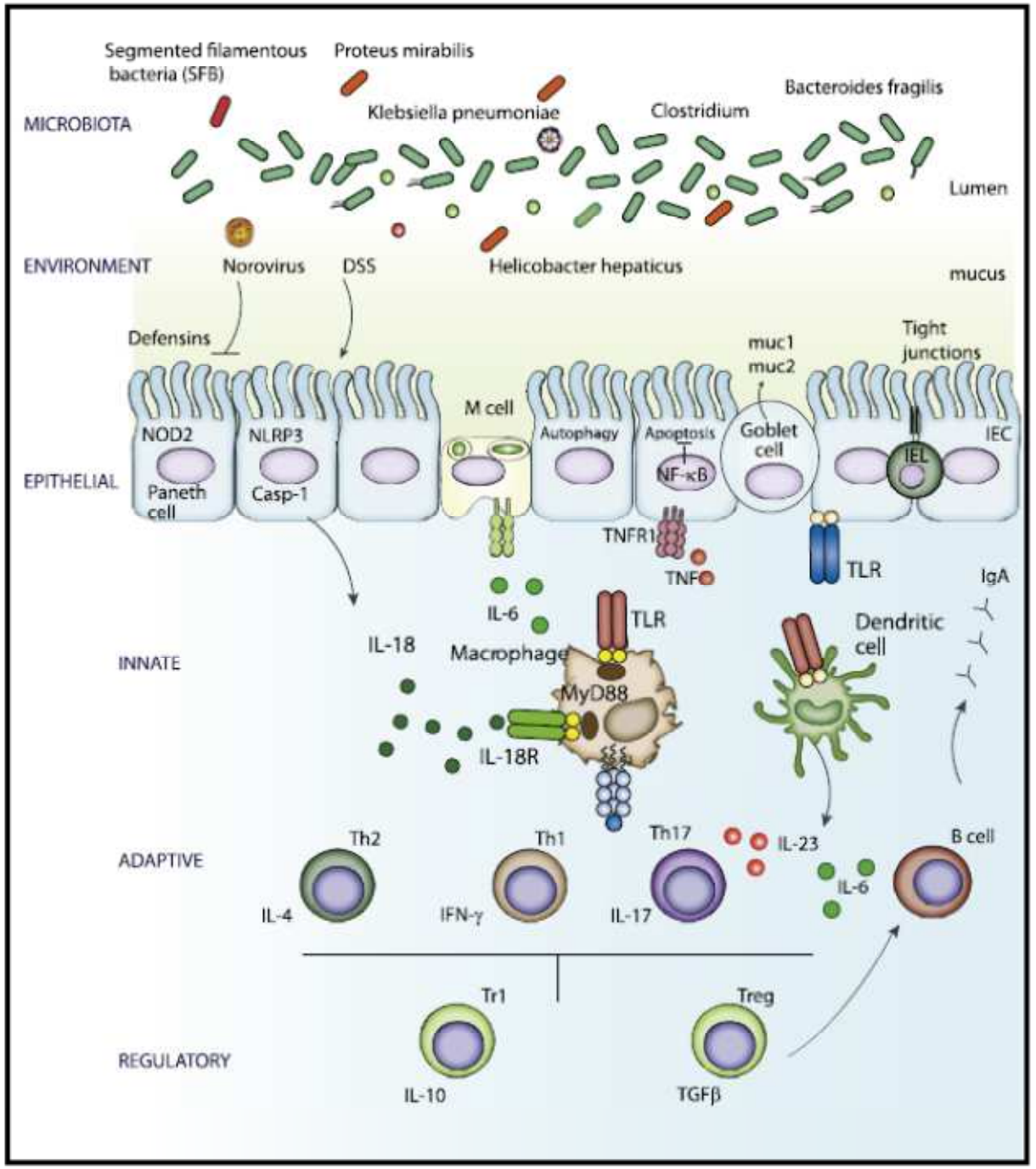
	Gene Involved	Mechanism	Microbiota or Environment
Spontaneous			
TRUC	*Tbx21*$^{-/-}$ *Rag2*$^{-/-}$	↑TNF (innate) ↓Treg (adaptive)	klebsiella pneumonia, proteus mirabilis
MUC2	*Muc2*$^{-/-}$	↓mucin (epithelial)	–
NEMO	*Ikbkg*^{IEC}	↑apoptosis (epithelial)	–
IKKα and IKKβ	*Ikbka*-*Ikbkb*^{IEC}	↑apoptosis (epithelial)	–
MDR1	*Abcb1b*$^{-/-}$	↑xenobiotic substances (epithelial)	–
TGF-β	*Tgfb1*$^{-/-}$	↓Treg (adaptive)	–
TGF-βRII	*Tgfbr2*$^{-/-}$	↓Treg (adaptive)	–
IL-10	*Il10*$^{-/-}$	↓Treg (adaptive)	–
IL-10R1	*Il10r1*$^{-/-}$	↓Treg (adaptive)	–
IL-2	*Il2*$^{-/-}$	↓Treg (adaptive)	–
FOXP3	*Foxp3*$^{-/-}$	↓Treg (adaptive)	–
Microbial			
H. hepaticus	–	↑Th17 (adaptive)	bacteroides fragilis (protective-↑IL-10-↓IL-17)
segmented filamentous bacteria (SFB)	–	↑Th17 (adaptive)	–
C. jejuni	*Muc1*	↓mucin (epithelial)	–
H. hepaticus	*Nod2*	↓defensin (epithelial) HD5^{IEC} transgenic (protective)	–

Table 1. Experimental Models of Colitis

Chemical			
DSS			
	Tnfaip3^{IEC}	↑apoptosis (epithelial)	–
	Tlr2$^{-/-}$, *Tlr4*$^{-/-}$	↓tissue repair (innate)	–
	Myd88$^{-/-}$	↓tissue repair (innate)	–
	Nlrp3$^{-/-}$	↓tissue repair (innate)	–
	Casp1$^{-/-}$	↓tissue repair (innate)	–
	Il18$^{-/-}$	↓tissue repair (innate)	–
	Il18r1$^{-/-}$	↓tissue repair (innate)	–
	Nod2$^{+/+}$	MDP protective, ↑tissue repair (innate)	–
	–		clostridium species (protective-↑TGF-β-↑Treg)
	Atg16l1$^{-/-}$	↑inflammasome (innate)	
	Atg16l1^{HM}	↓Paneth cell function	norovirus + microbiota
Immune			
$CD4^+CD45^+Rb^{(hi)}$ transfer			
	Mylk transgenic	disrupted tight junctions (epithelial)	–
	Stat4 transgenic	↑Th1 (adaptive)	
	–	IL-23 neutralization, protective ↓Th17 (adaptive)	–
	–	$CD4^+CD25^+$ cotransfer (↑Treg), protective (adaptive)	–
microbiota-reactive memory $CD4^+$ Th1 cells transfer	–	↑Th1 (adaptive)	
microbiota-reactive memory $CD4^+$ Th17 cells transfer	–	↑Th17 (adaptive)	

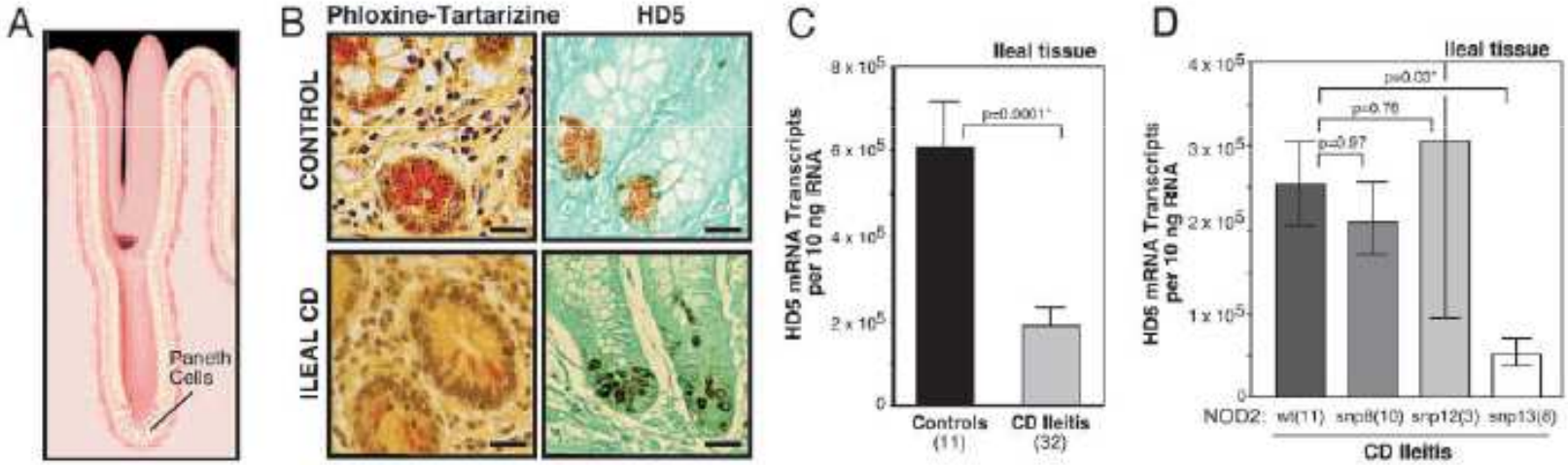


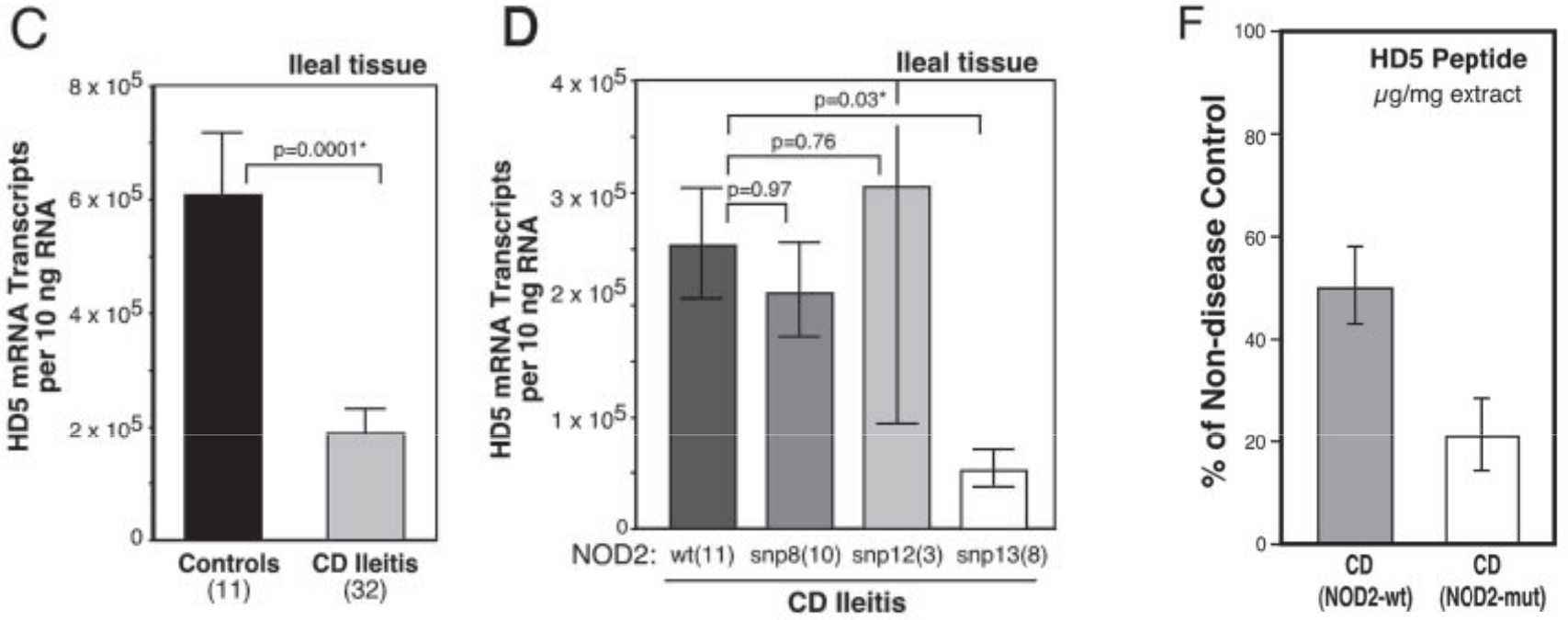
TGF-β	*Tgfb1*$^{-/-}$	↓Treg (adaptive)
TGF-βRII	*Tgfbr2*$^{-/-}$	↓Treg (adaptive)
IL-10	*Il10*$^{-/-}$	↓Treg (adaptive)
IL-10R1	*Il10r1*$^{-/-}$	↓Treg (adaptive)
IL-2	*Il2*$^{-/-}$	↓Treg (adaptive)
FOXP3	*Foxp3*$^{-/-}$	↓Treg (adaptive)


Segmented filamentous bacteria (SFB)
Proteus mirabilis
Klebsiella pneumoniae
Clostridium
Bacteroides fragilis
MICROBIOTA
Lumen
ENVIRONMENT
Norovirus
DSS
Helicobacter hepaticus
mucus
Defensins
muc1
muc2
Tight junctions
M cell
Autophagy
Apoptosis
Goblet cell
IEC
IEL
NOD2
NLRP3
EPITHELIAL
Paneth cell
Casp-1
NF-κB
TNFR1
TNF
TLR
IgA
IL-6
TLR
IL-18
Macrophage
Dendritic cell
INNATE
MyD88
IL-18R
Th2
Th1
Th17
IL-23
B cell
ADAPTIVE
IL-4
IFN-γ
IL-17
IL-6
Tr1
Treg
REGULATORY
IL-10
TGFβ

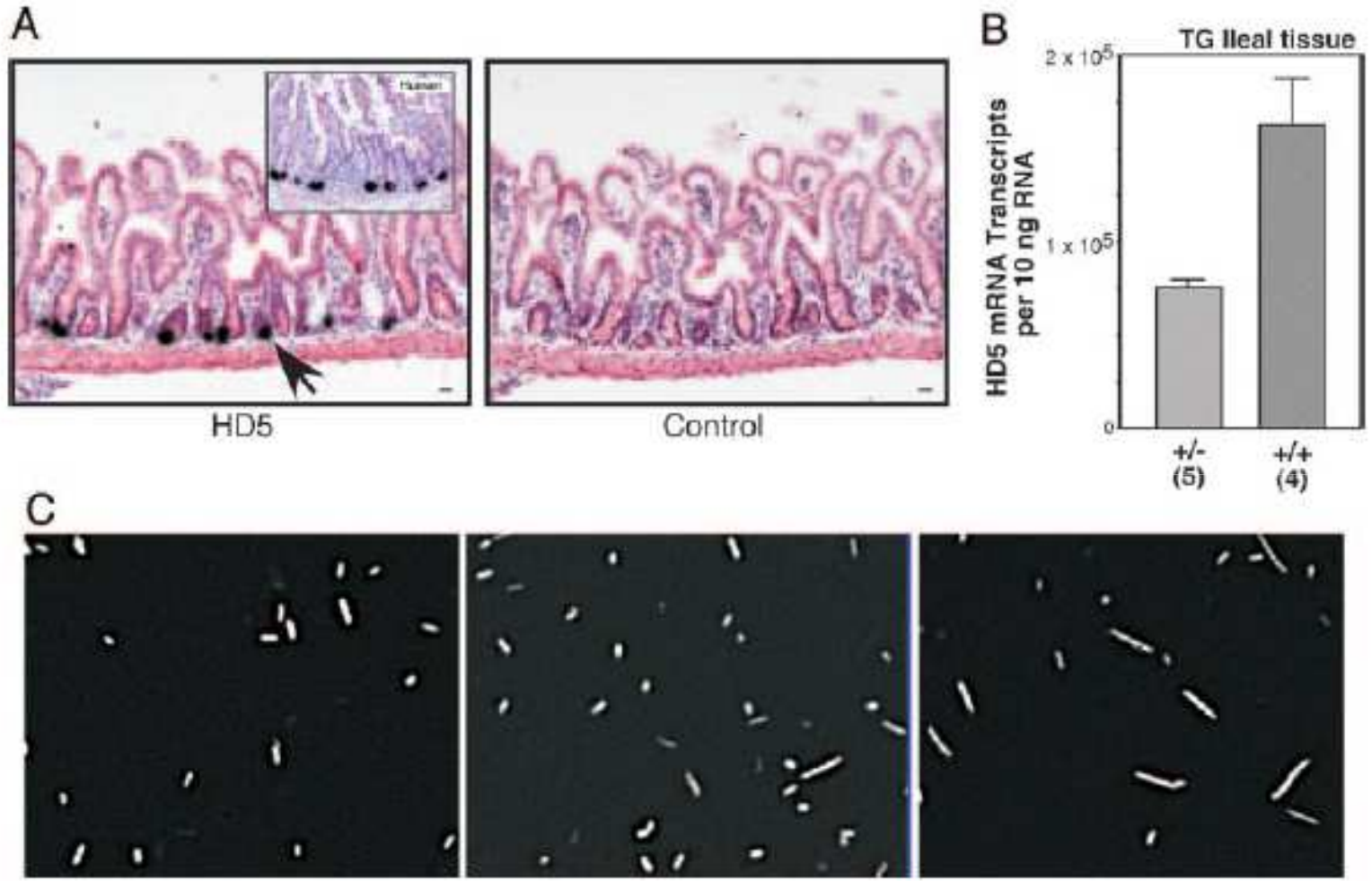
Reduced Paneth cell α-defensins in ileal Crohn's disease

Jan Wehkamp*, Nita H. Salzman†, Edith Porter‡§, Sabine Nuding¶‖, Michael Weichenthal**, Robert E. Petras††, Bo Shen‡‡, Elke Schaeffeler‖, Matthias Schwab‖, Rose Linzmeier§, Ryan W. Feathers*, Hiutung Chu*, Heriberto Lima, Jr.‡, Klaus Fellermann¶‖, Tomas Ganz§, Eduard F. Stange¶‖§§, and Charles L. Bevins*§§




C
Ileal tissue
HD5 mRNA Transcripts per 10 ng RNA
8 x 10^5
6 x 10^5
4 x 10^5
2 x 10^5
0
p=0.0001*
Controls (11)
CD Ileitis (32)
D
Ileal tissue
HD5 mRNA Transcripts per 10 ng RNA
4 x 10^5
3 x 10^5
2 x 10^5
1 x 10^5
0
p=0.03*
p=0.76
p=0.97
NOD2: wt(11) snp8(10) snp12(3) snp13(8)
CD Ileitis
F
HD5 Peptide
μg/mg extract
% of Non-disease Control
100
80
60
40
20
0
CD (NOD2-wt)
CD (NOD2-mut)

HD5 TG Mice, modello murino che sovraesprime HD5 (human defensin 5)



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IKKα and IKKβ	*Ikbka*-*Ikbkb*^{IEC}	↑apoptosis (epithelial)	–
MDR1	*Abcb1b*$^{-/-}$	↑xenobiotic substances (epithelial)	–
TGF-β	*Tgfb1*$^{-/-}$	↓Treg (adaptive)	–
TGF-βRII	*Tgfbr2*$^{-/-}$	↓Treg (adaptive)	–
IL-10	*Il10*$^{-/-}$	↓Treg (adaptive)	–
IL-10R1	*Il10r1*$^{-/-}$	↓Treg (adaptive)	–
IL-2	*Il2*$^{-/-}$	↓Treg (adaptive)	–
FOXP3	*Foxp3*$^{-/-}$	↓Treg (adaptive)	–
Microbial			
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	Il18$^{-/-}$	↓tissue repair (innate)	–
	Il18r1$^{-/-}$	↓tissue repair (innate)	–
	Nod2$^{+/+}$	MDP protective, ↑tissue repair (innate)	–
	–		clostridium species (protective-↑TGF-β-↑Treg)
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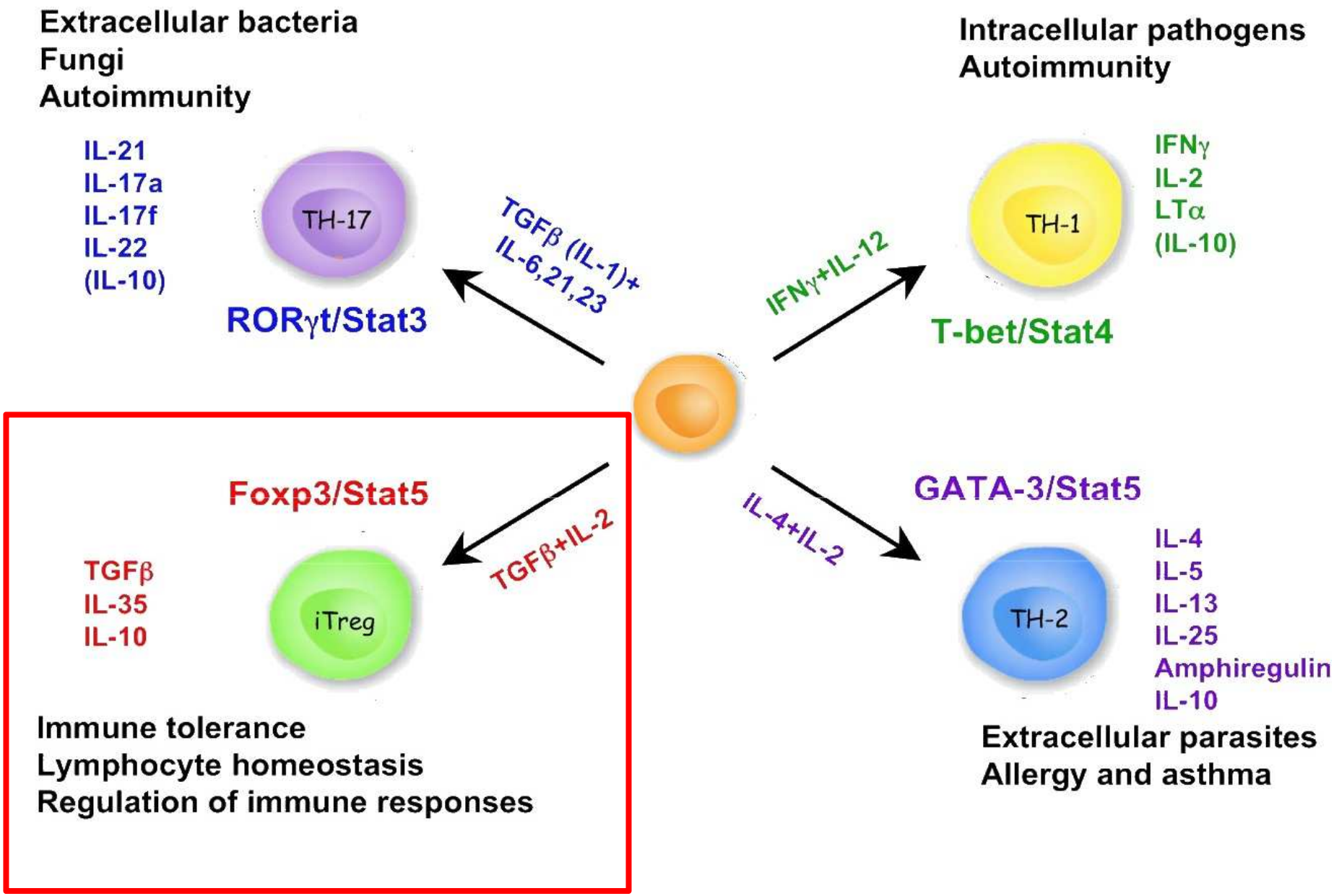
CD4^{+}CD45^{+}Rb$^{(hi)}$ transfer			
	Mylk transgenic	disrupted tight junctions (epithelial)	–
	Stat4 transgenic	↑Th1 (adaptive)	
	–	IL-23 neutralization, protective ↓Th17 (adaptive)	–
	–	CD4^{+}CD25^{+} cotransfer (↑Treg), protective (adaptive)	–
microbiota-reactive memory CD4^{+} Th1 cells transfer	–	↑Th1 (adaptive)	
microbiota-reactive memory CD4^{+} Th17 cells transfer	–	↑Th17 (adaptive)	


Extracellular bacteria
Fungi
Autoimmunity
IL-21
IL-17a
IL-17f
IL-22
(IL-10)
TH-17
RORγt/Stat3
TGFβ (IL-1)+
IL-6,21,23
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TH-1
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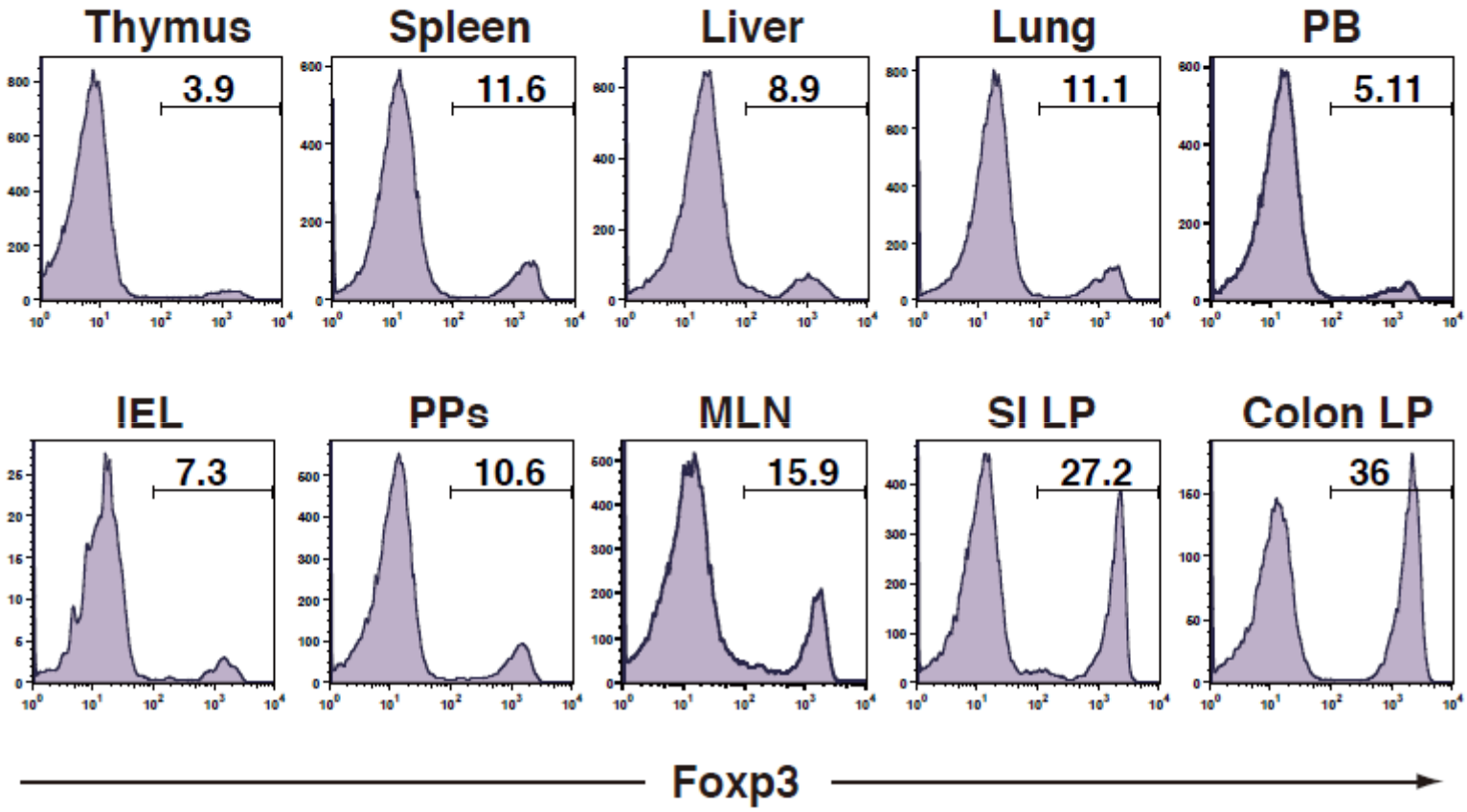
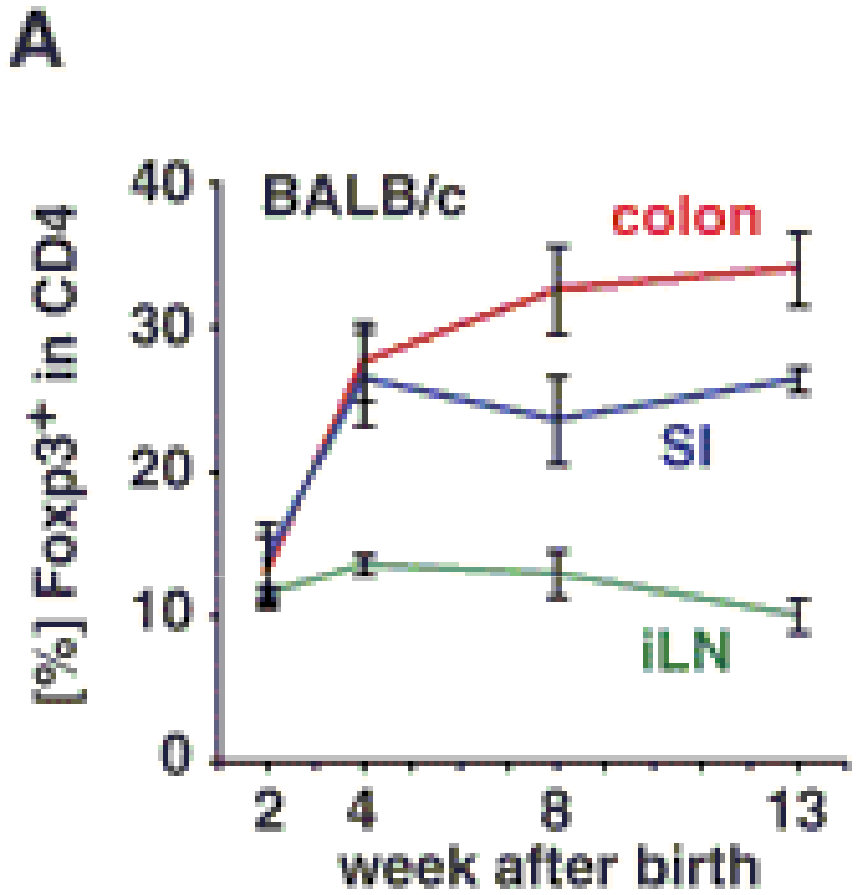

Thymus
3.9
Spleen
11.6
Liver
8.9
Lung
11.1
PB
5.11
IEL
7.3
PPs
10.6
MLN
15.9
SI LP
27.2
Colon LP
36
Foxp3

Induction of Colonic Regulatory T Cells by Indigenous *Clostridium* Species

Koji Atarashi,[1*] Takeshi Tanoue,[1*] Tatsuichiro Shima,[2] Akemi Imaoka,[2] Tomomi Kuwahara,[3] Yoshika Momose,[4] Genhong Cheng,[5] Sho Yamasaki,[6] Takashi Saito,[6] Yusuke Ohba,[7] Tadatsugu Taniguchi,[1] Kiyoshi Takeda,[8] Shohei Hori,[9] Ivaylo I. Ivanov,[10] Yoshinori Umesaki,[2] Kikuji Itoh,[4] Kenya Honda[1,11]†

$CD4^+$ T regulatory cells (T_{regs}), which express the Foxp3 transcription factor, play a critical role in the maintenance of immune homeostasis. Here, we show that in mice, T_{regs} were most abundant in the colonic mucosa. The spore-forming component of indigenous intestinal microbiota, particularly clusters IV and XIVa of the genus *Clostridium*, promoted T_{reg} cell accumulation. Colonization of mice by a defined mix of *Clostridium* strains provided an environment rich in transforming growth factor–β and affected $Foxp3^+$ T_{reg} number and function in the colon. Oral inoculation of *Clostridium* during the early life of conventionally reared mice resulted in resistance to colitis and systemic immunoglobulin E responses in adult mice, suggesting a new therapeutic approach to autoimmunity and allergy.

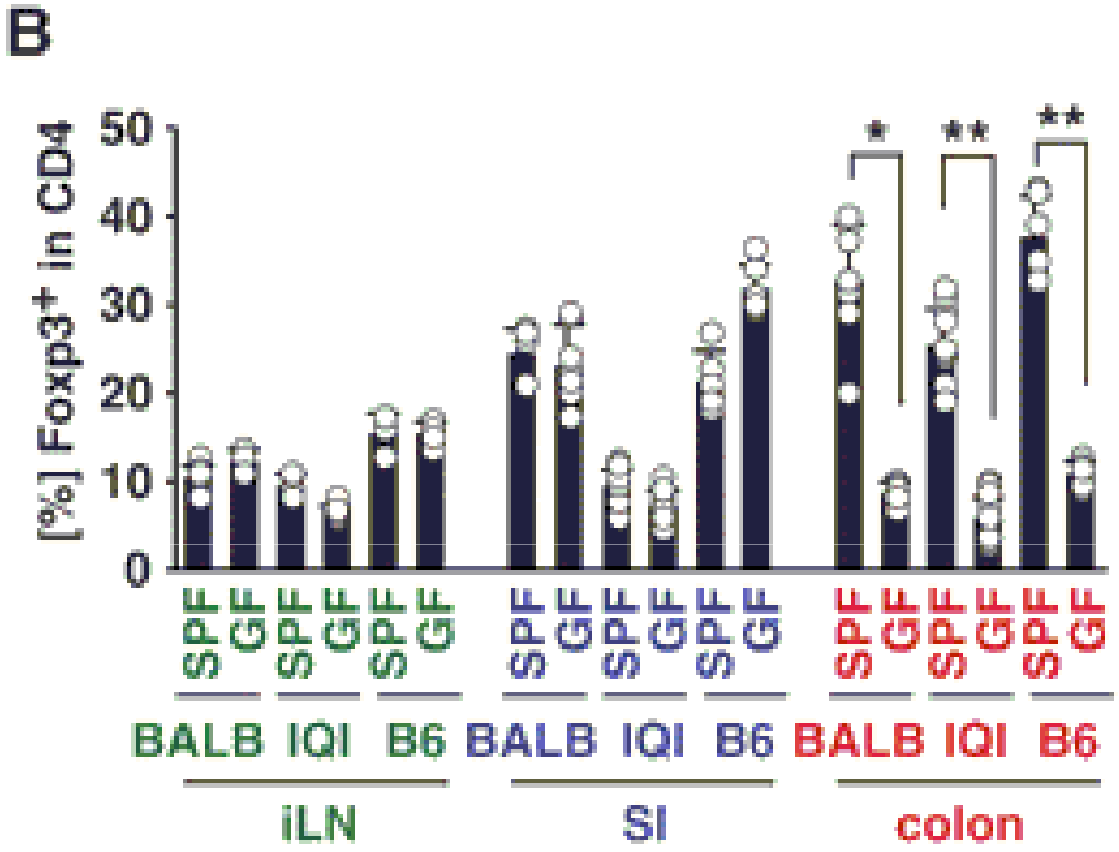


Indigenous intestinal bacteria-dependent accumulation of colonic Tregs. (A) The percentage of Foxp3+ cells within the CD4+ cell population isolated from iLNs or LP of colon or SI of SPF BALB/c mice at the indicated age was analyzed by flow cytometry.

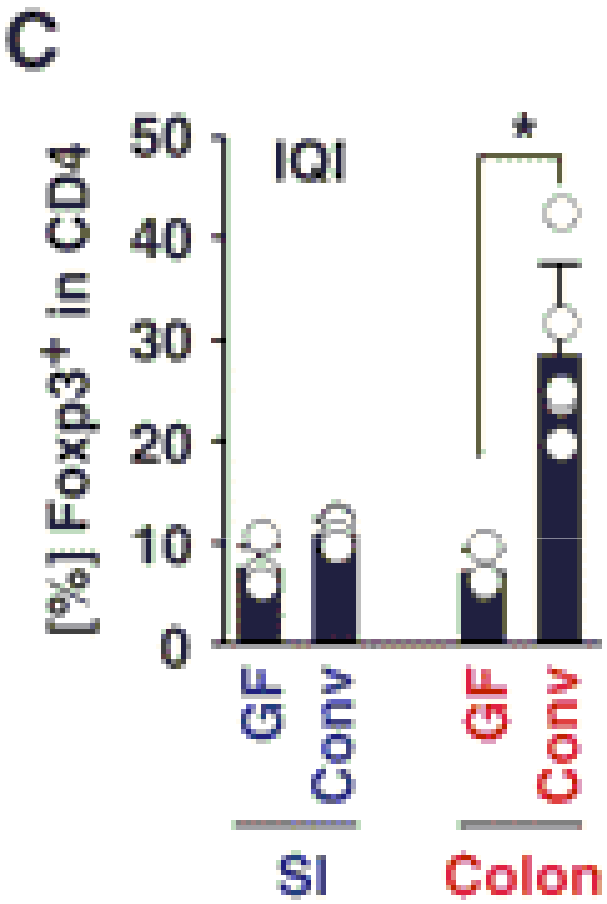
A **gnotobiotic animal** (from Greek roots *gnostos* 'known' and *bios* 'life') is an animal in which only certain known strains of bacteria and other microorganisms are present. Technically, the term also includes germ-free animals, as the status of their microbial communities is also *known.*

Germ-free animals are animals that have no microorganisms living in or on it. Such animals are raised within germ-free isolators in order to control their exposure to viral, bacterial or parasitic agents.

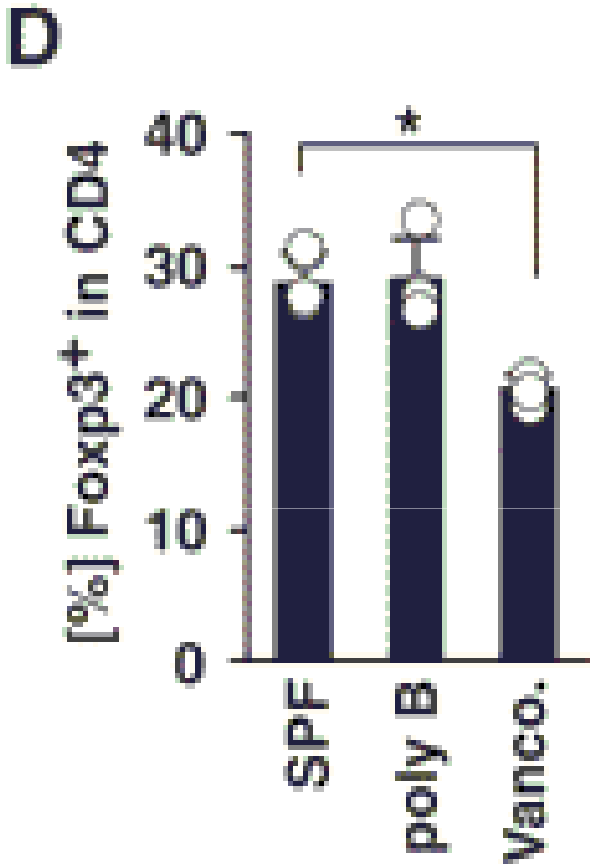
Specific Pathogen Free is a term used for laboratory animals that are guaranteed free of particular pathogens. Use of SPF animals ensures that specified diseases do not interfere with an experiment. For example, absence of respiratory pathogens such as influenza is desirable when investigating a drug's effect on lung function.



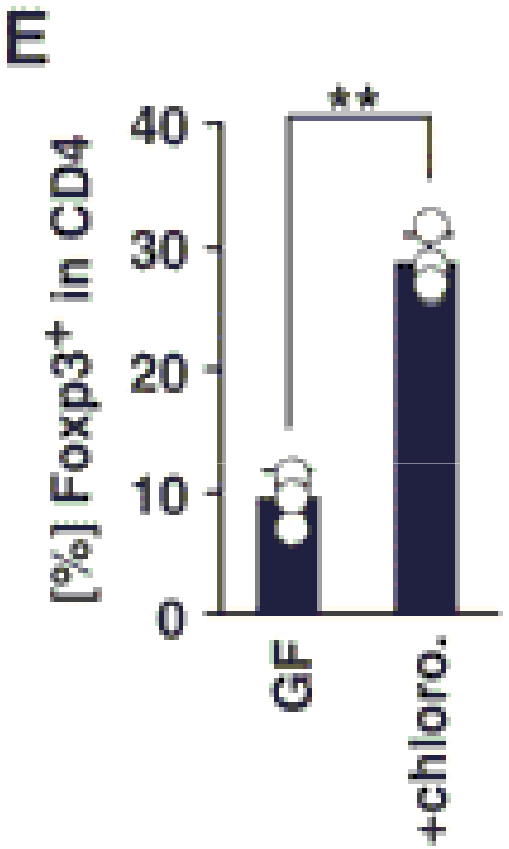
(B) Lymphocytes from SI, colon, and iLN of 8-week-old BALB/c, IQI and C57BL/6 (B6) GF, and SPF mice were analyzed for CD4 and Foxp3 expression



C) GF IQI mice were conventionalized (Conv) by oral administration of the fecal suspension from B6 SPF mice purchased from Jackson Laboratory. Colonic LP lymphocytes were isolated 3 weeks later and analyzed for Foxp3 expression. (



(D) Four-week-old SPF B6 mice were treated with polymyxin B (poly B) or vancomycin (Vanco) for 4 weeks and analyzed for the percentage of Foxp3+ cells within the CD4+ cell population.



(E) GF mice were gavaged with chloroform-treated feces from SPF mice (+chloro) and analyzed for the percentage of Foxp3+ cells within the CD4+ cell population. Each circle in (B) to (E) represents an individual mouse, and error bars indicate the SD. Data were obtained from more than two independent experiments with similar results (n ≥ 4 mice per group).

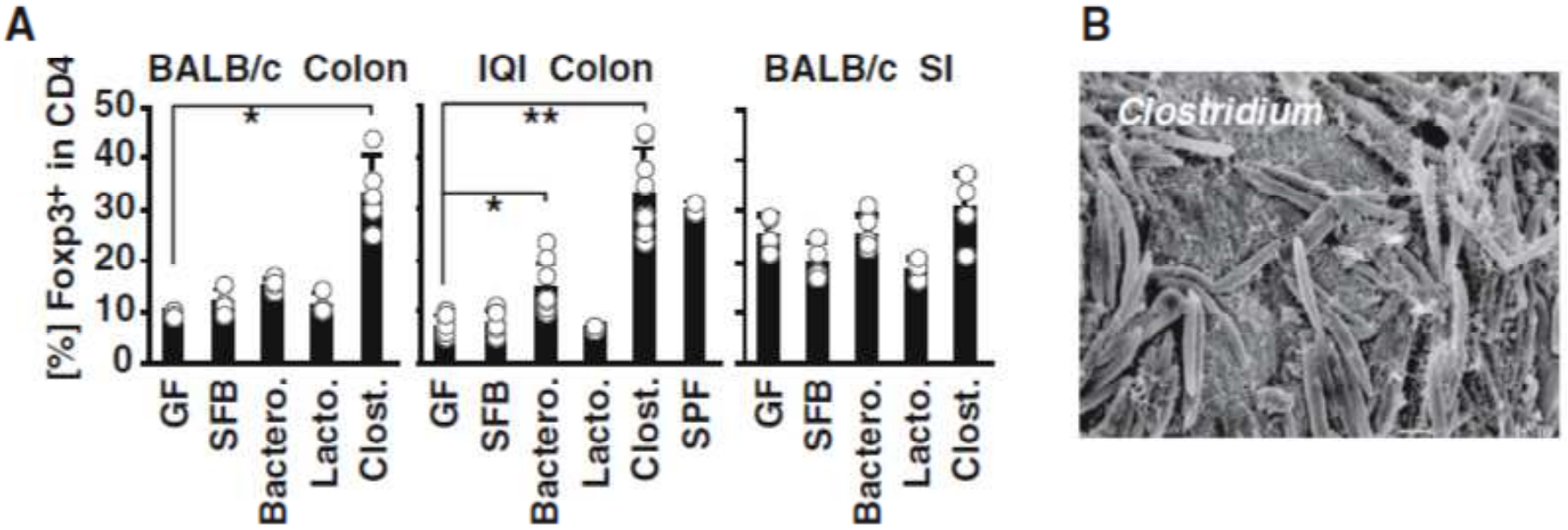


Fig. 2. Clostridia induce T_{reg} accumulation in colonic LP. (**A**) GF BALB/c or IQI mice were colonized with segmented filamentous bacteria (SFB), 16 strains of *Bacteroides* (Bactero.), 3 strains of *Lactobacillus* (Lacto.), or 46 strains of *Clostridium* (Clost.) for 3 weeks. The percentage of $Foxp3^+$ cells within the $CD4^+$ cell population in the colon and SI of individual mice was analyzed by flow cytometry ($n \geq 5$ mice per group). (**B**) Electron micrograph showing the proximal colon of Clost.-colonized B6 mice. (**C**) Lymphocytes

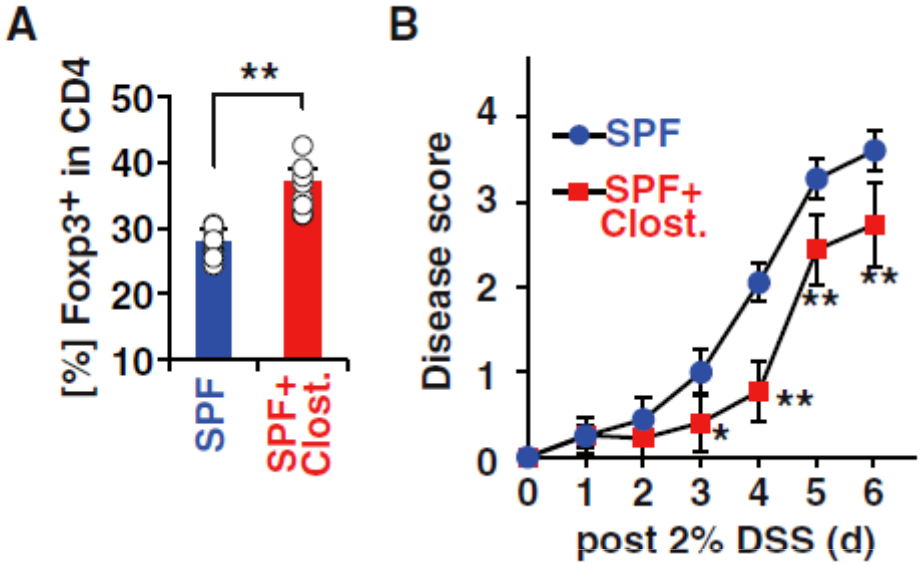
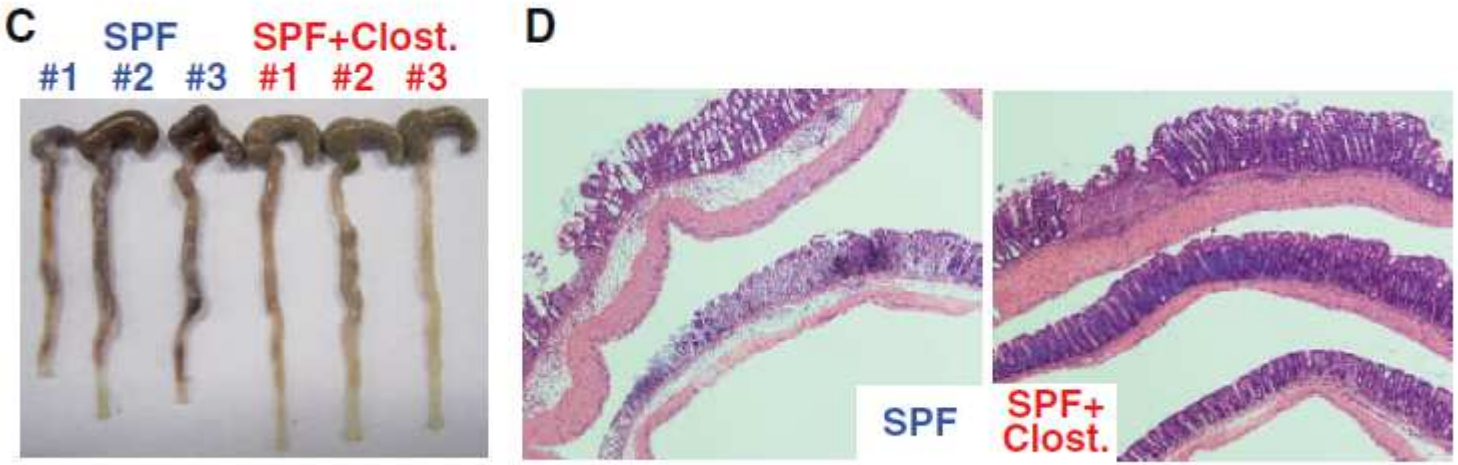
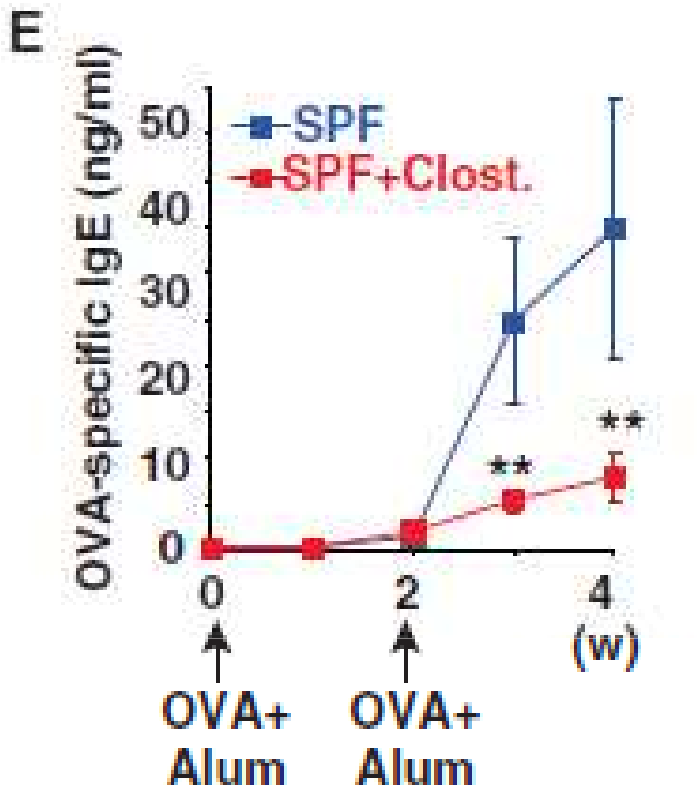
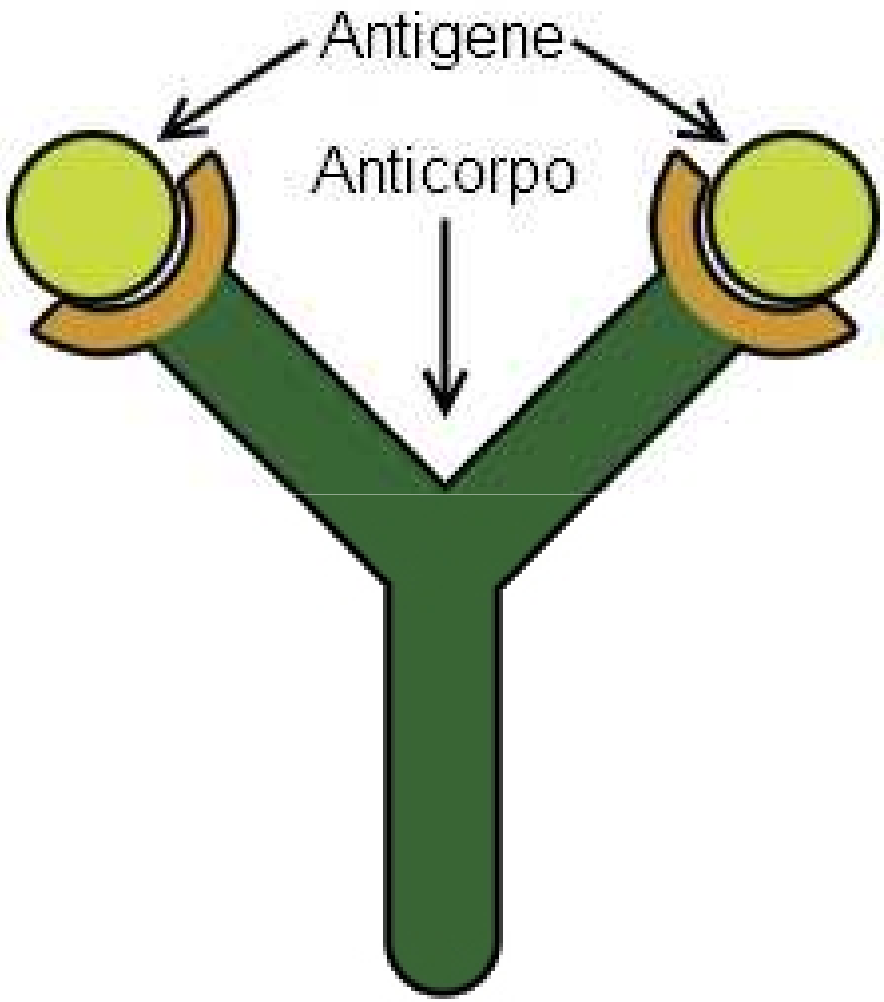


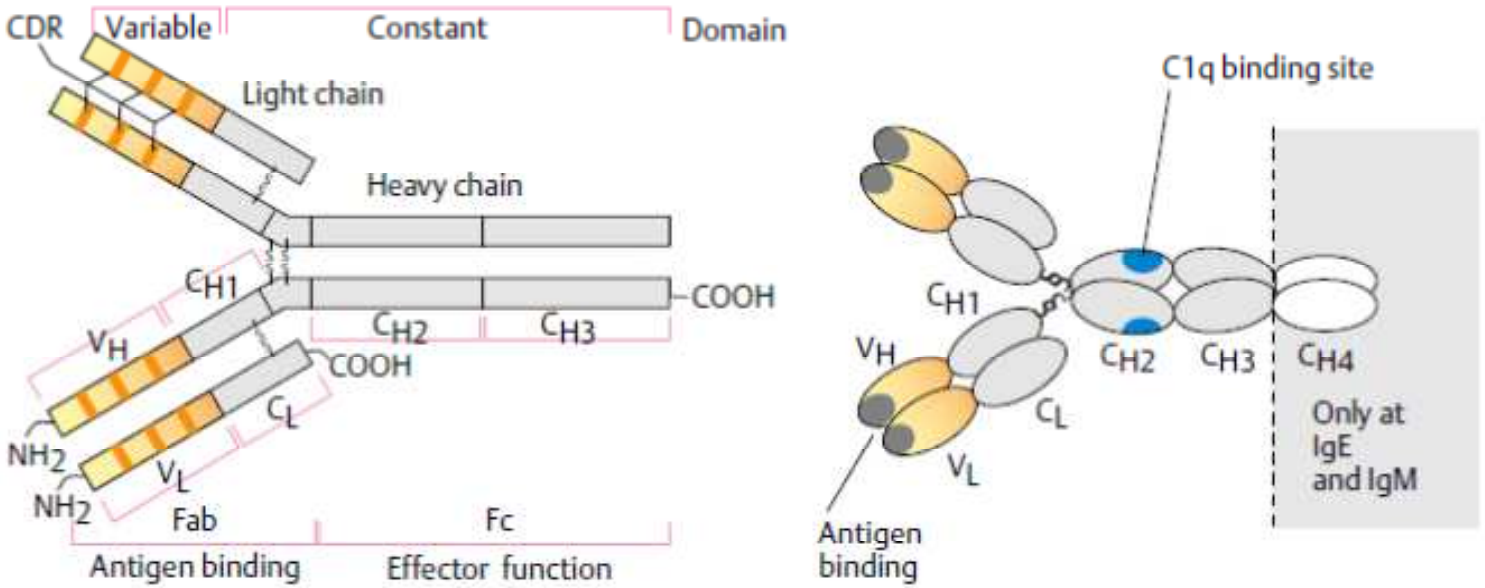
Fig. 4. Effect of *Clostridium* abundance on regulation of colitis and IgE response. (**A**) Two-week-old SPF mice were orally inoculated with *Clostridium* (SPF+Clost.) or untreated (SPF). After 6 weeks, the percentage of Foxp3$^+$ cells within CD4$^+$ cells in colonic LP was analyzed. The experiment was repeated more than three times with similar results. Each circle represents a mouse, and error bars represent the SD (n = 7 mice per group). (**B** to **D**) SPF and SPF+Clost. mice were treated with 2% DSS and monitored and scored for body weight loss, stool consistency, and bleeding for 6 days (n = 7 mice per group). (B) On day 6, the colons were collected (C) and dissected for histological analysis by hematoxylin and





(E and F) SPF and SPF+Clost. mice were immunized with OVA + alum twice at a 2-week interval. Sera were collected and examined for OVA-specific IgE levels by ELISA (E).

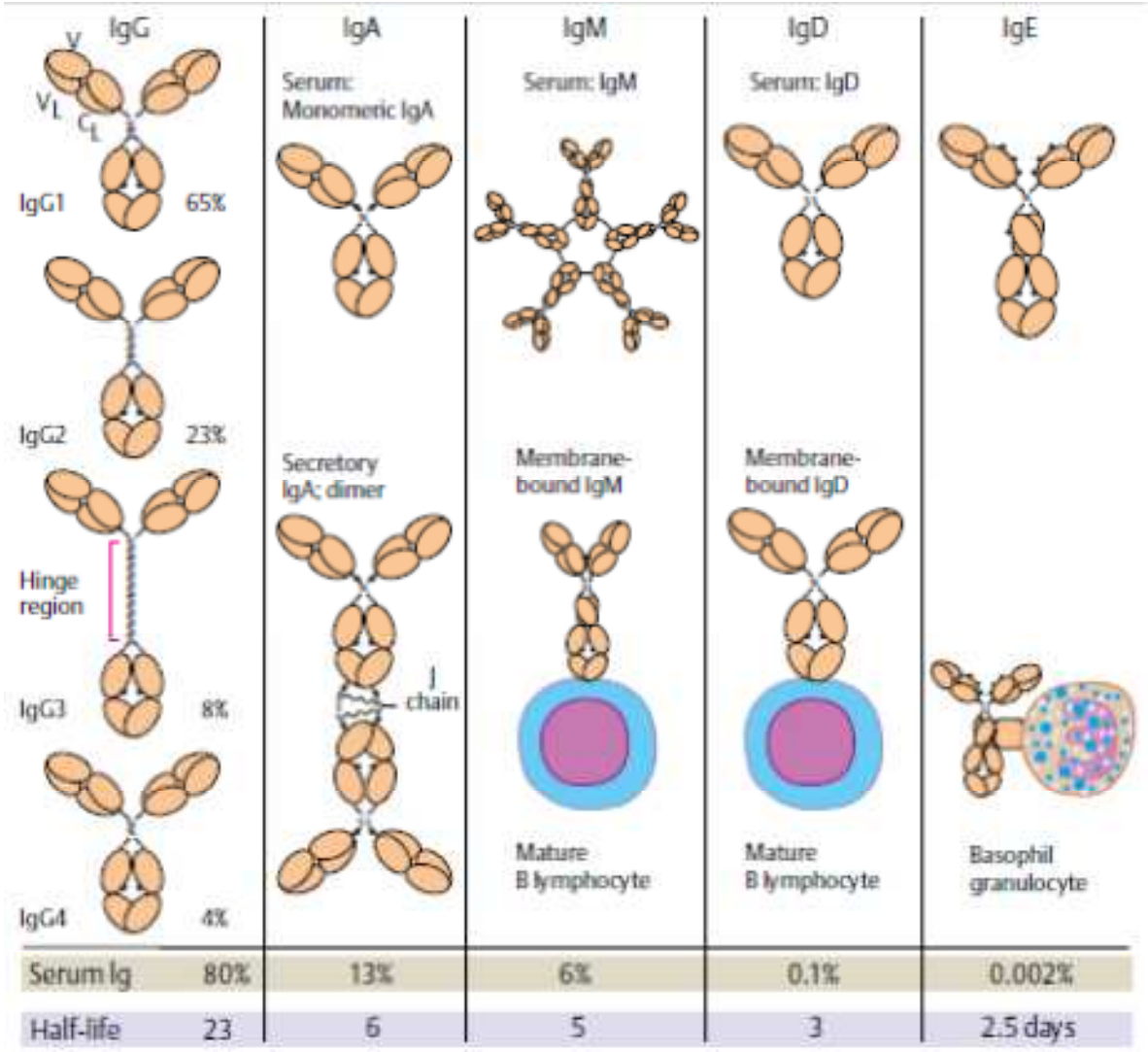

Antigene
Anticorpo



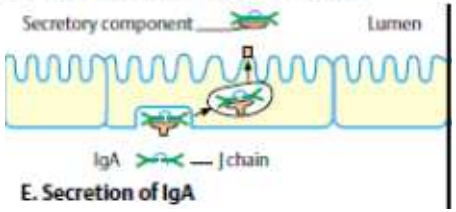
CDR = complementarity-determining region
Fab = antigen-binding fragment
Fc = crystallizable fragment

V_H = variable domain of heavy chains
V_L = variable domain of light chains
$C_{H/L}$ = constant domain of heavy / light chains

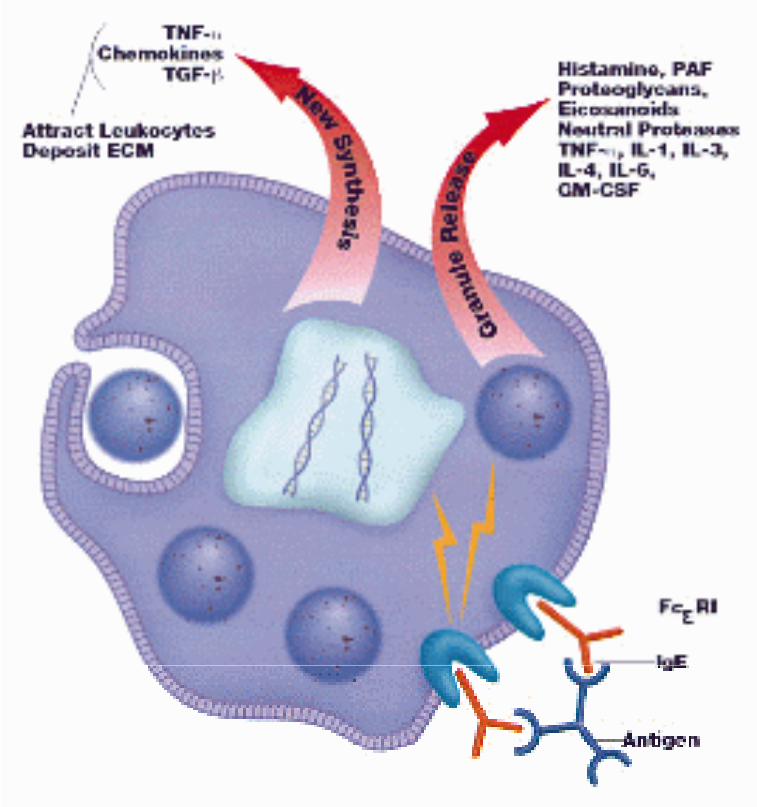
A. Immunoglobulin structure



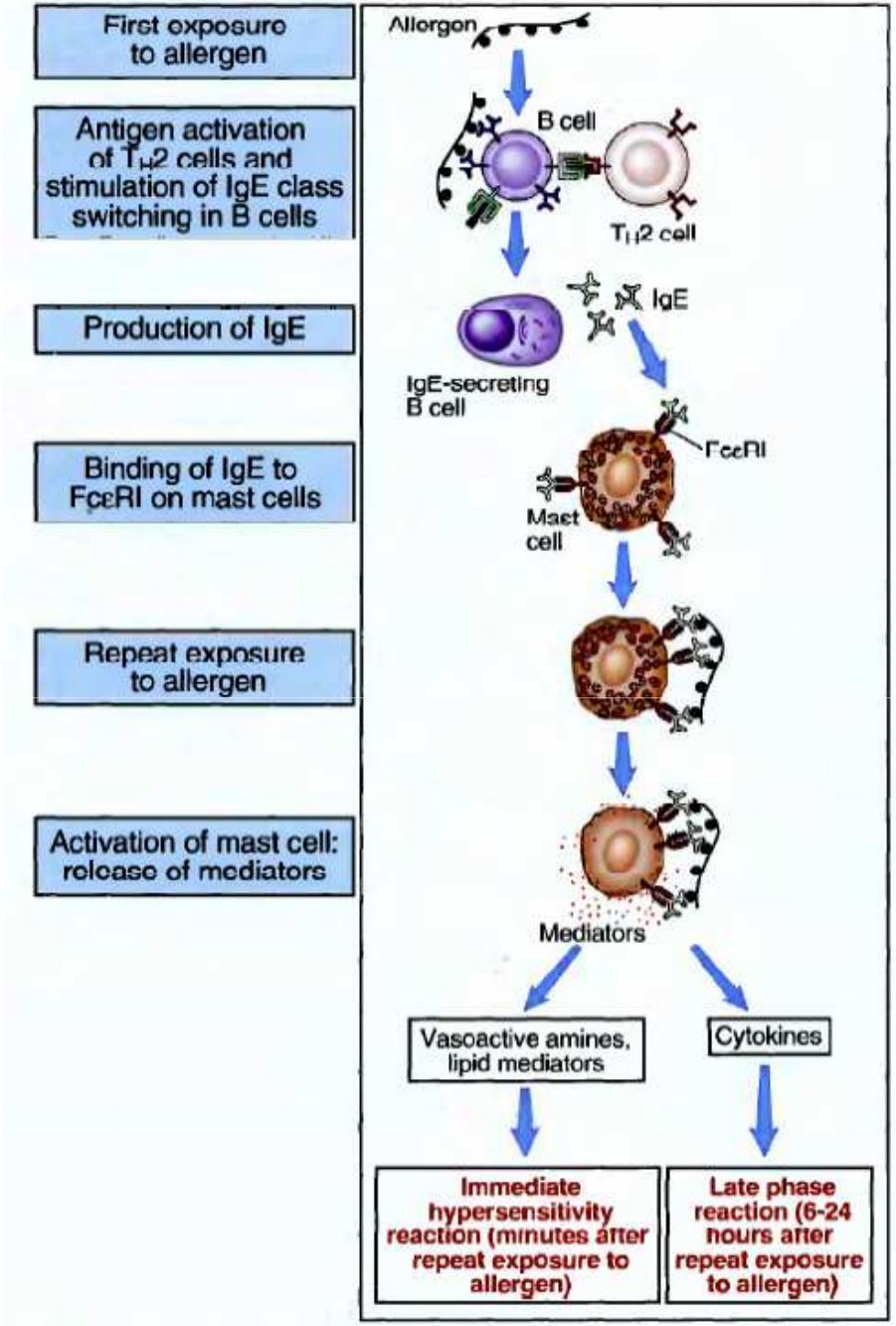
C. Immunglobulin structure and features

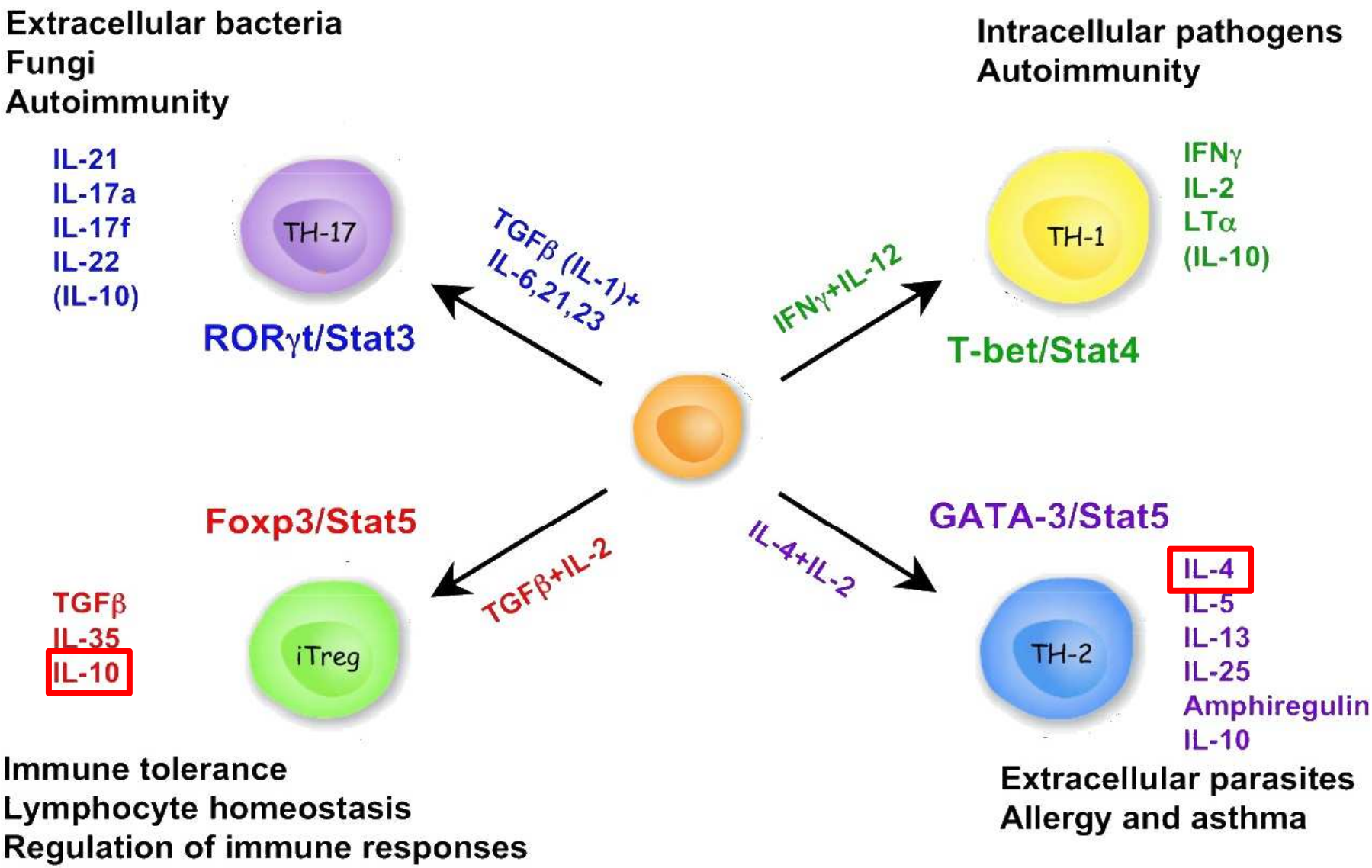


E. Secretion of IgA

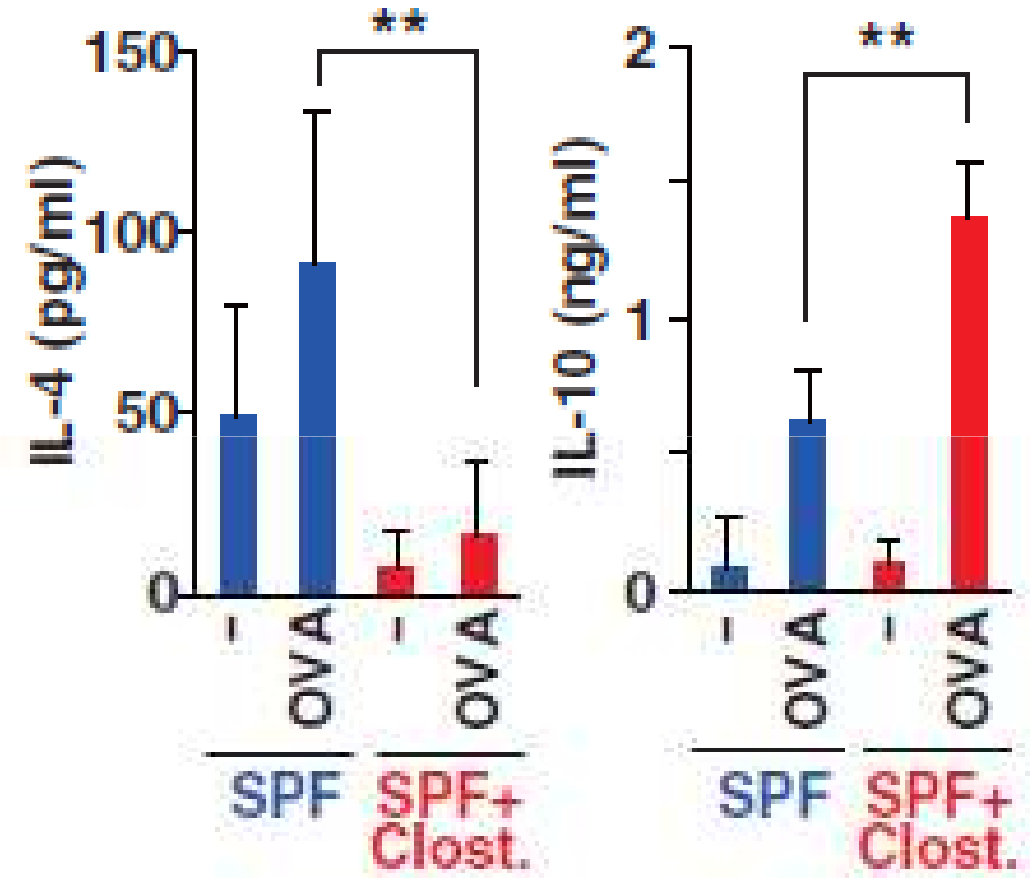


Le IgE sono presenti nel siero in modesta concentrazione, sono formate da due catene pesanti chiamate ε, 20000 dalton più pesanti delle catene γ delle IgG e di conseguenza presentano un ulteriore dominio. Le IgE sono responsabili delle allergie pertanto possono trovarsi negli individui allergici in elevata concentrazione. L'ulteriore dominio permette il legame alla superficie delle mastcellule, ciò comporta una reazione che porta alla liberazione di sostanze farmacologicamente attive come istamina e serotonina causa di dilatazione capillare, alterazione della permeabilità e costrizione bronchiale


First exposure to allergen
Antigen activation of T_H2 cells and stimulation of IgE class switching in B cells
Production of IgE
Binding of IgE to FcεRI on mast cells
Repeat exposure to allergen
Activation of mast cell: release of mediators
Allergen
B cell
T_H2 cell
IgE
IgE-secreting B cell
FcεRI
Mast cell
Mediators
Vasoactive amines, lipid mediators
Cytokines
Immediate hypersensitivity reaction (minutes after repeat exposure to allergen)
Late phase reaction (6-24 hours after repeat exposure to allergen)


Extracellular bacteria
Fungi
Autoimmunity
IL-21
IL-17a
IL-17f
IL-22
(IL-10)
TH-17
RORγt/Stat3
TGFβ (IL-1)+
IL-6,21,23
IFNγ+IL-12
TH-1
T-bet/Stat4
Intracellular pathogens
Autoimmunity
IFNγ
IL-2
LTα
(IL-10)
Foxp3/Stat5
TGFβ
IL-35
IL-10
iTreg
TGFβ+IL-2
IL-4+IL-2
GATA-3/Stat5
TH-2
IL-4
IL-5
IL-13
IL-25
Amphiregulin
IL-10
Immune tolerance
Lymphocyte homeostasis
Regulation of immune responses
Extracellular parasites
Allergy and asthma

F


**
150
100
50
0
IL-4 (pg/ml)
-
OVA
-
OVA
SPF
SPF+
Clost.
**
2
1
0
IL-10 (ng/ml)
-
OVA
-
OVA
SPF
SPF+
Clost.

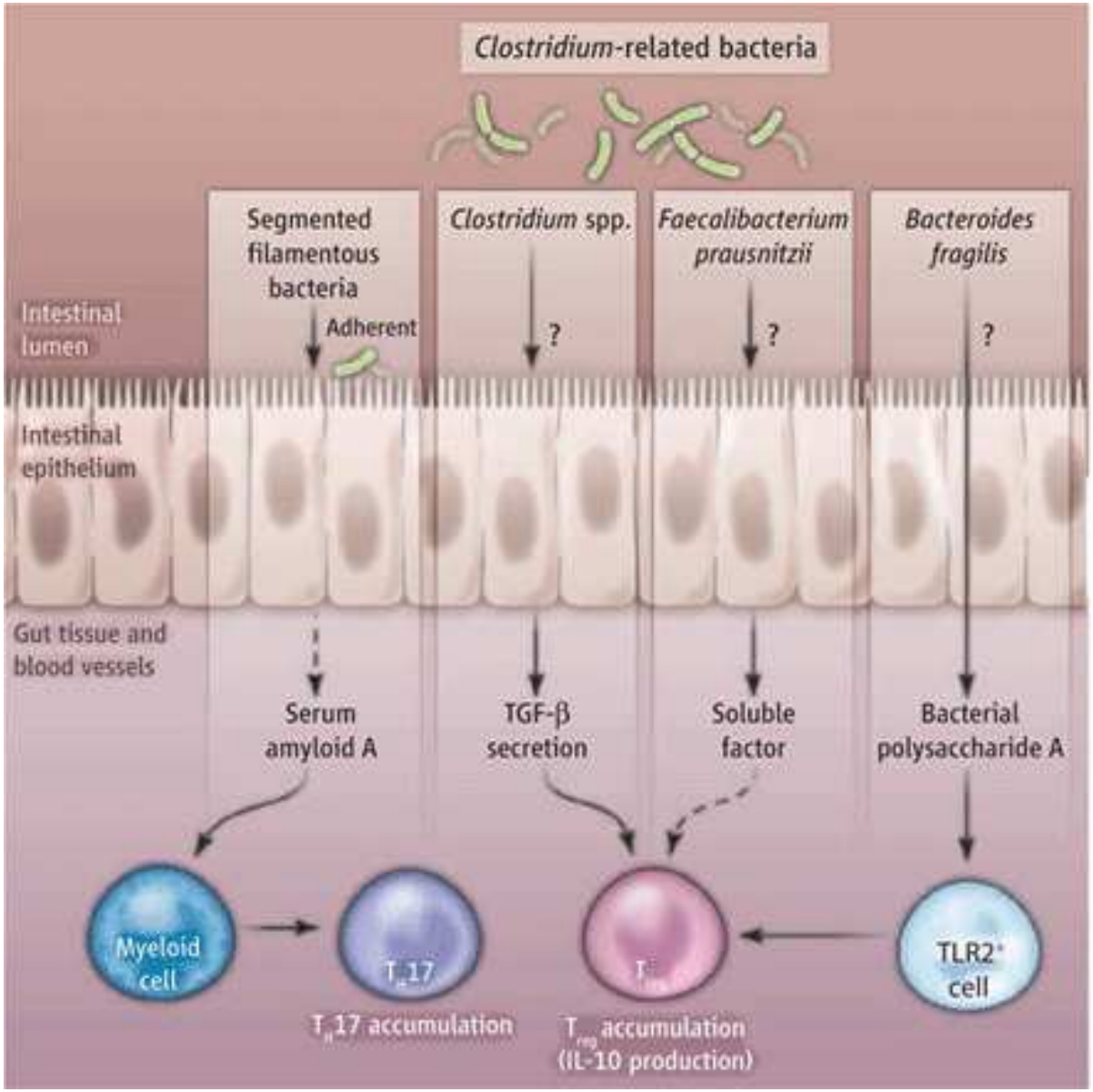
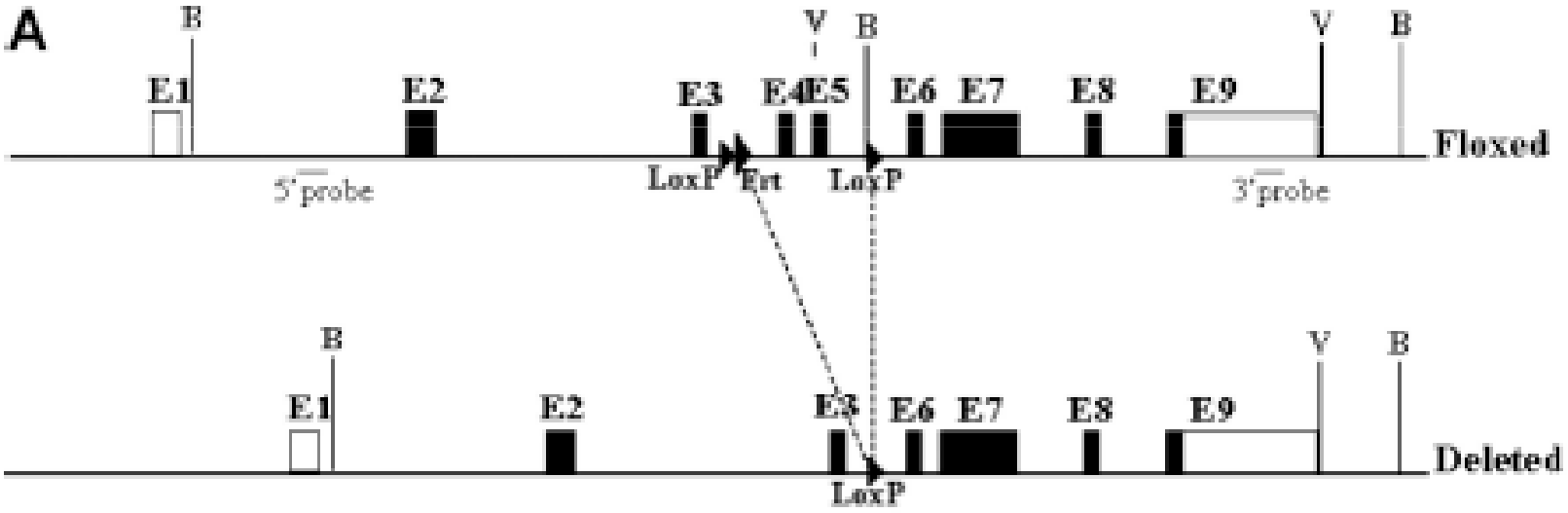

Clostridium-related bacteria
Segmented filamentous bacteria
Clostridium spp.
Faecalibacterium prausnitzii
Bacteroides fragilis
Intestinal lumen
Adherent
?
?
?
Intestinal epithelium
Gut tissue and blood vessels
Serum amyloid A
TGF-β secretion
Soluble factor
Bacterial polysaccharide A
Myeloid cell
T_H17
T_{reg}
TLR2+ cell
T_H17 accumulation
T_{reg} accumulation (IL-10 production)

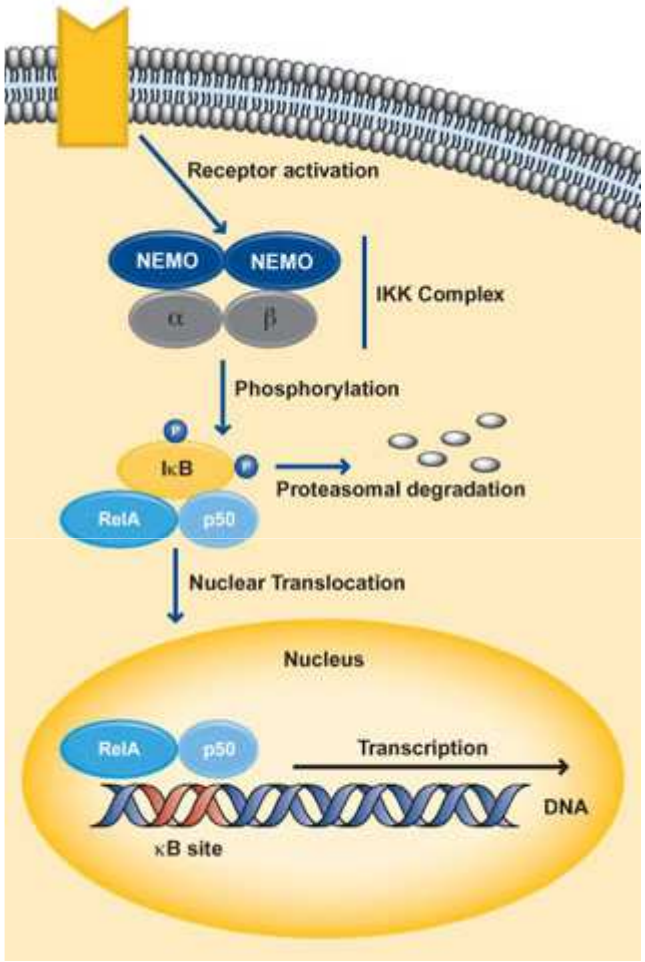
Table 1. Experimental Models of Colitis

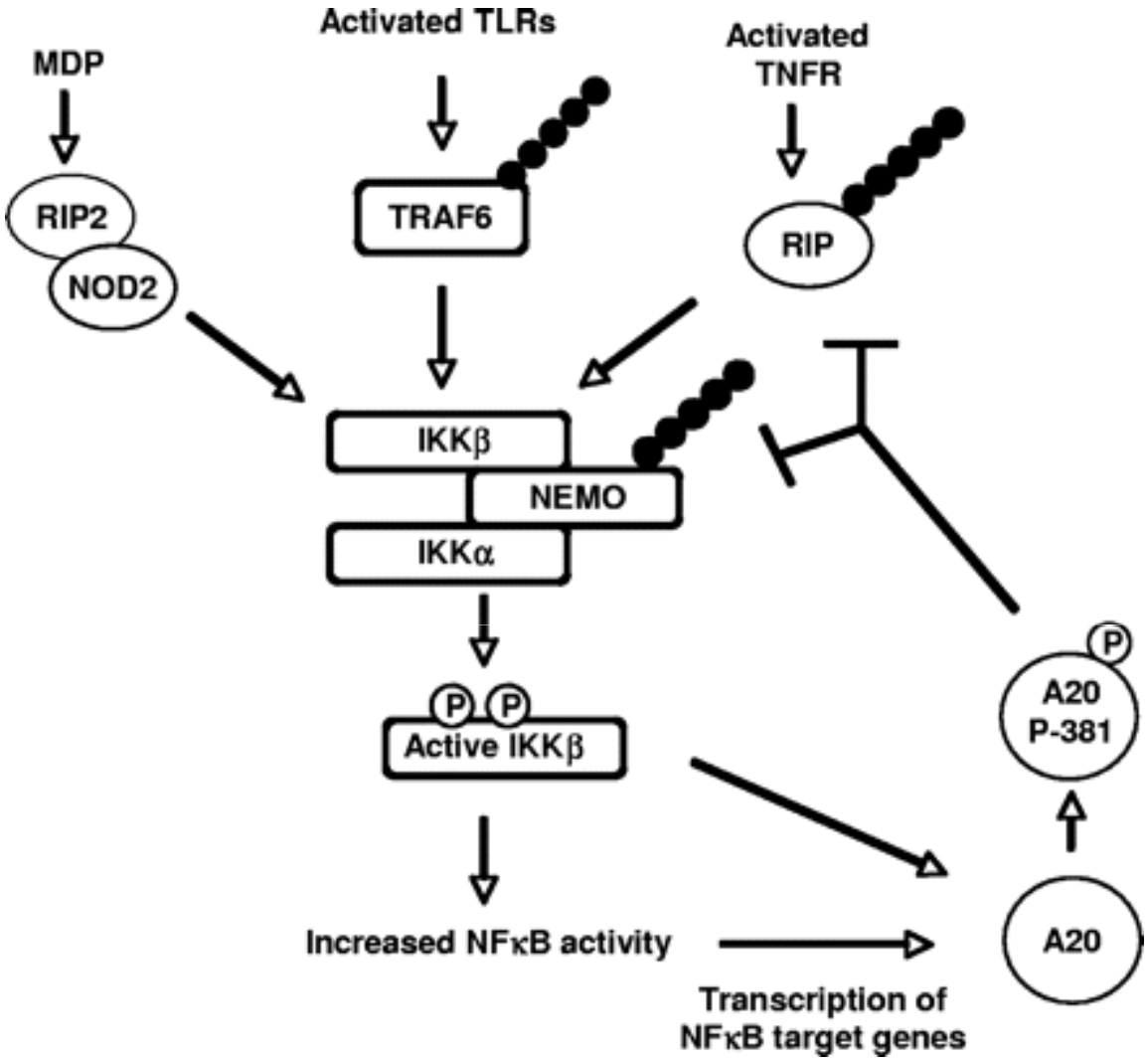
Chemical			
DSS			
	Tnfaip3^{IEC}	↑apoptosis (epithelial)	–
	Tlr2$^{-/-}$, *Tlr4*$^{-/-}$	↓tissue repair (innate)	–
	Myd88$^{-/-}$	↓tissue repair (innate)	–
	Nlrp3$^{-/-}$	↓tissue repair (innate)	–
	Casp1$^{-/-}$	↓tissue repair (innate)	–
	Il18$^{-/-}$	↓tissue repair (innate)	–
	Il18r1$^{-/-}$	↓tissue repair (innate)	–
	Nod2$^{+/+}$	MDP protective, ↑tissue repair (innate)	–
	–		clostridium species (protective-↑TGF-β-↑Treg)
	Atg16l1$^{-/-}$	↑inflammasome (innate)	
	Atg16l1^{HM}	↓Paneth cell function	norovirus + microbiota
Immune			
$CD4^+CD45^+Rb^{(hi)}$ transfer			
	Mylk transgenic	disrupted tight junctions (epithelial)	–
	Stat4 transgenic	↑Th1 (adaptive)	
	–	IL-23 neutralization, protective ↓Th17 (adaptive)	–
	–	$CD4^+CD25^+$ cotransfer (↑Treg), protective (adaptive)	–
microbiota-reactive memory $CD4^+$ Th1 cells transfer	–	↑Th1 (adaptive)	
microbiota-reactive memory $CD4^+$ Th17 cells transfer	–	↑Th17 (adaptive)	

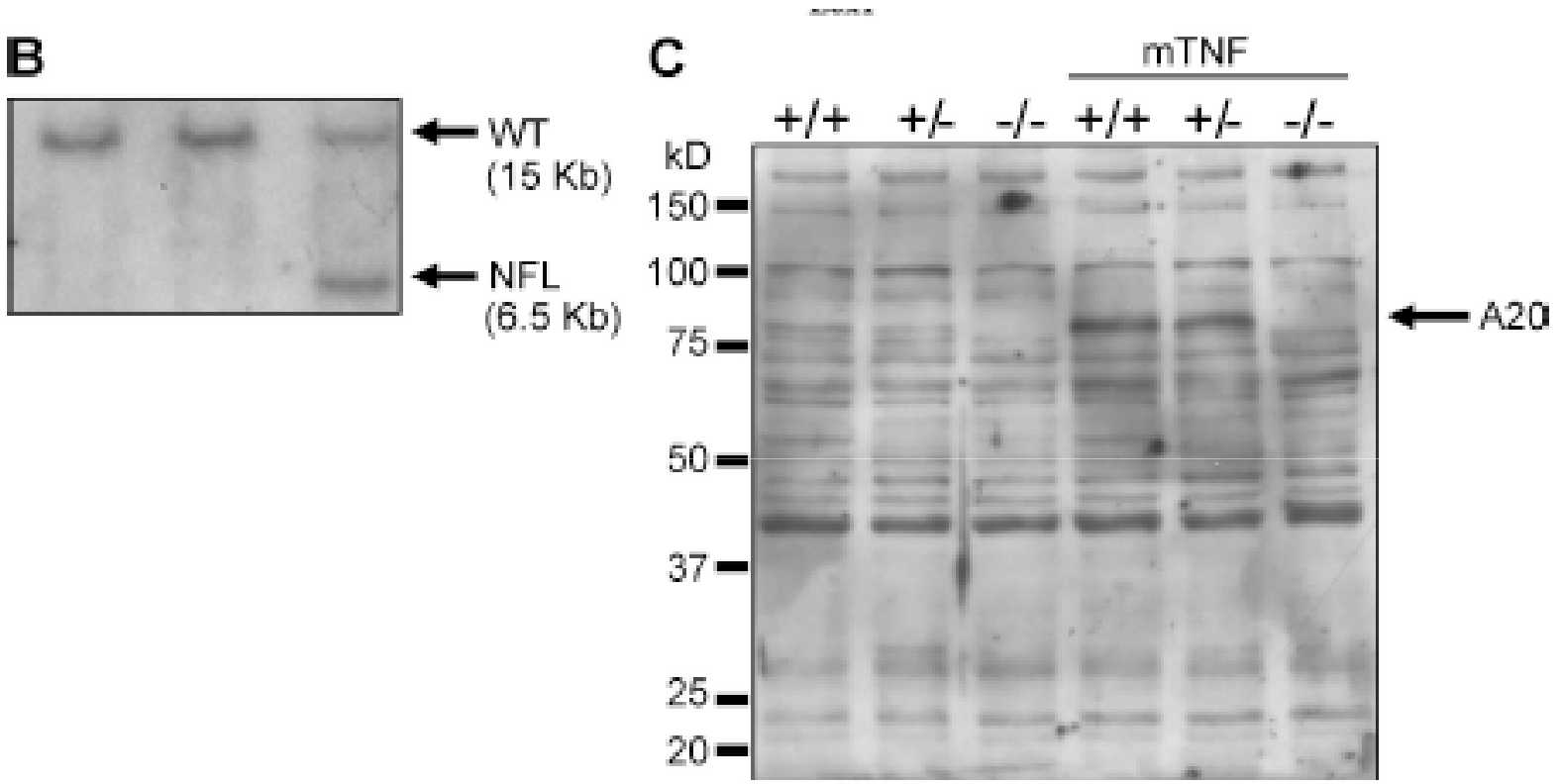
Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor–induced toxicity and experimental colitis

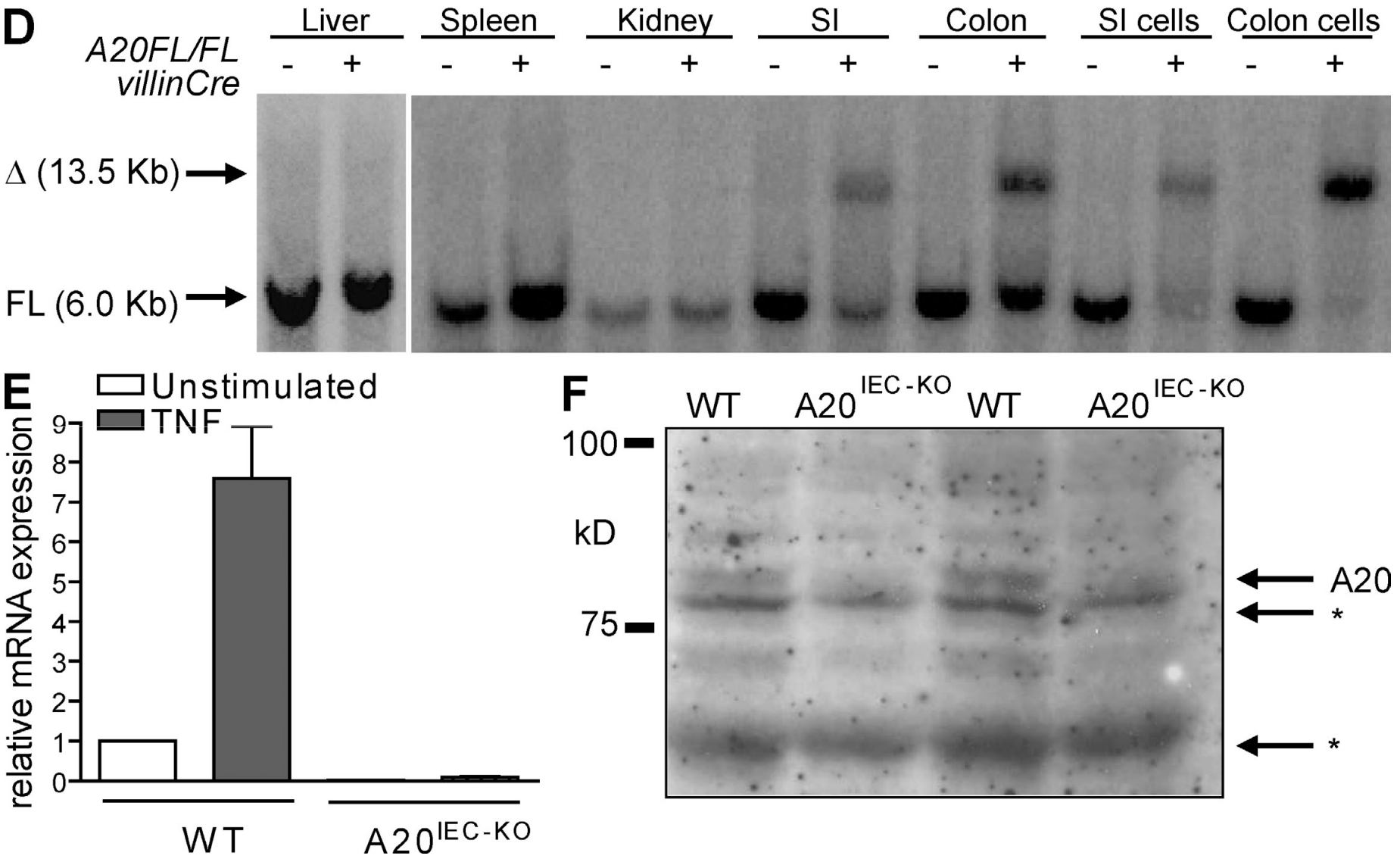
Lars Vereecke,[1,2] Mozes Sze,[1,2] Conor Mc Guire,[1,2] Brecht Rogiers,[1,2]
Yuanyuan Chu,[3] Marc Schmidt-Supprian,[3] Manolis Pasparakis,[4]
Rudi Beyaert,[1,2] and Geert van Loo[1,2]




Receptor activation
NEMO
NEMO
IKK Complex
α
β
Phosphorylation
P
IκB
P
Proteasomal degradation
RelA
p50
Nuclear Translocation
Nucleus
RelA
p50
Transcription
DNA
κB site


MDP
Activated TLRs
Activated
TNFR
RIP2
NOD2
TRAF6
RIP
IKKβ
NEMO
IKKα
P
P
Active IKKβ
P
A20
P-381
A20
Increased NFκB activity
Transcription of
NFκB target genes


B
WT
(15 Kb)
NFL
(6.5 Kb)
C
mTNF
+/+
+/-
-/-
+/+
+/-
-/-
kD
150
100
75
50
37
25
20
A20


D
A20FL/FL
villinCre
Liver
Spleen
Kidney
SI
Colon
SI cells
Colon cells
- + - + - + - + - + - + - +
Δ (13.5 Kb)
FL (6.0 Kb)
E
Unstimulated
TNF
relative mRNA expression
0 1 2 3 4 5 6 7 8 9
WT
A20IEC-KO
F
WT
A20IEC-KO
WT
A20IEC-KO
100
kD
75
A20
*
*

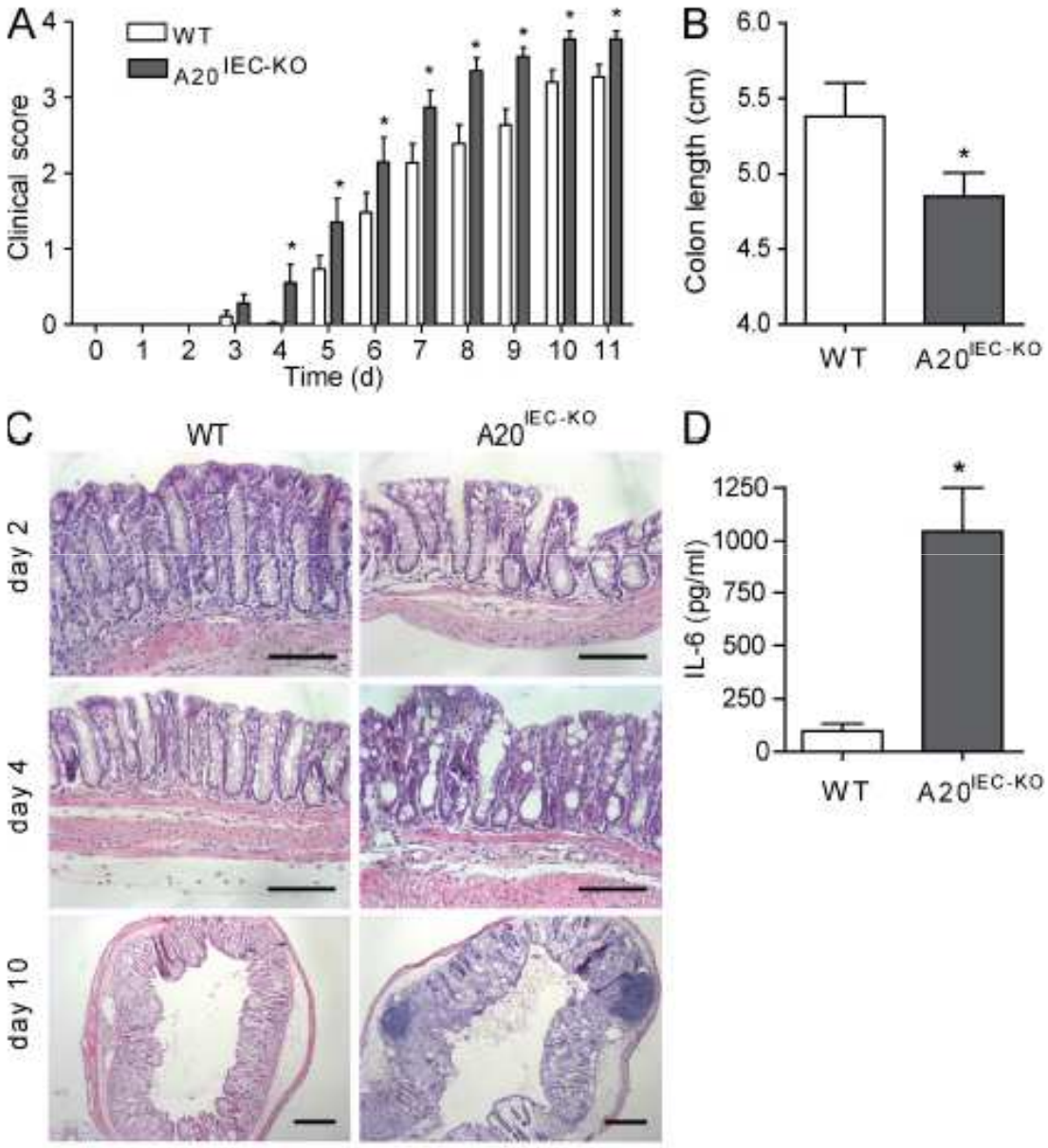
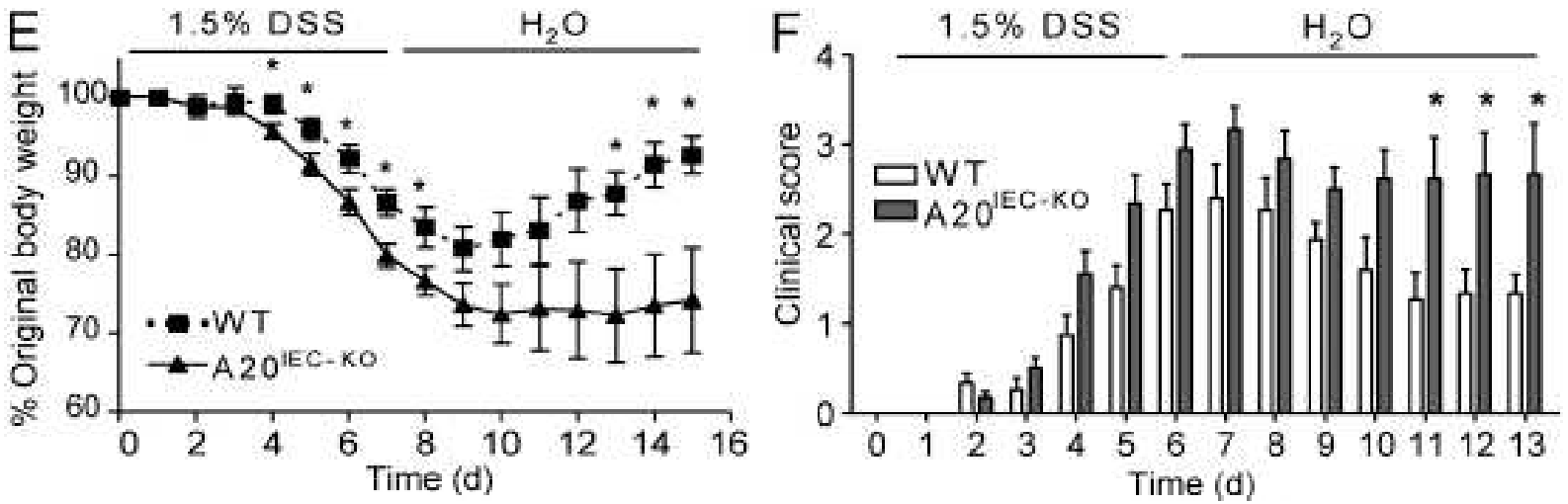


Figure 2. Enterocyte expression of A20 is required for recovery of mice from acute DSS-induced inflammation. (A) Clinical score of $A20^{IEC\text{-}KO}$ mice ($n = 18$) and control littermates (WT, $n = 20$) treated with 1.5% DSS. (B) Colon length of $A20^{IEC\text{-}KO}$ mice ($n = 18$) and control littermates (WT, $n = 20$) after 10 d of 1.5% DSS treatment. (C) Hematoxylin and eosin (H&E) histology on DSS-treated $A20^{IEC\text{-}KO}$ mice and control littermates (WT). Bars: (days 2 and 4) 100 µm; (day 10) 250 µm. (D) Serum IL-6 levels after 1.5% DSS treatment for 4 d. (E and F) Clinical

C
WT
A20IEC-KO
day 2
day 4
day 10



1.5% DSS treatment for 4 d. (E and F) Clinical score and body weight of $A20^{IEC-KO}$ mice ($n = 6$) and control littermates (WT, $n = 5$) treated for 6 d with 1.5% DSS followed by normal drinking water. Data in A–D are representative of three independent experiments. Experiments in E and F were performed two times. Error bars represent SEM. *, $P < 0.05$.

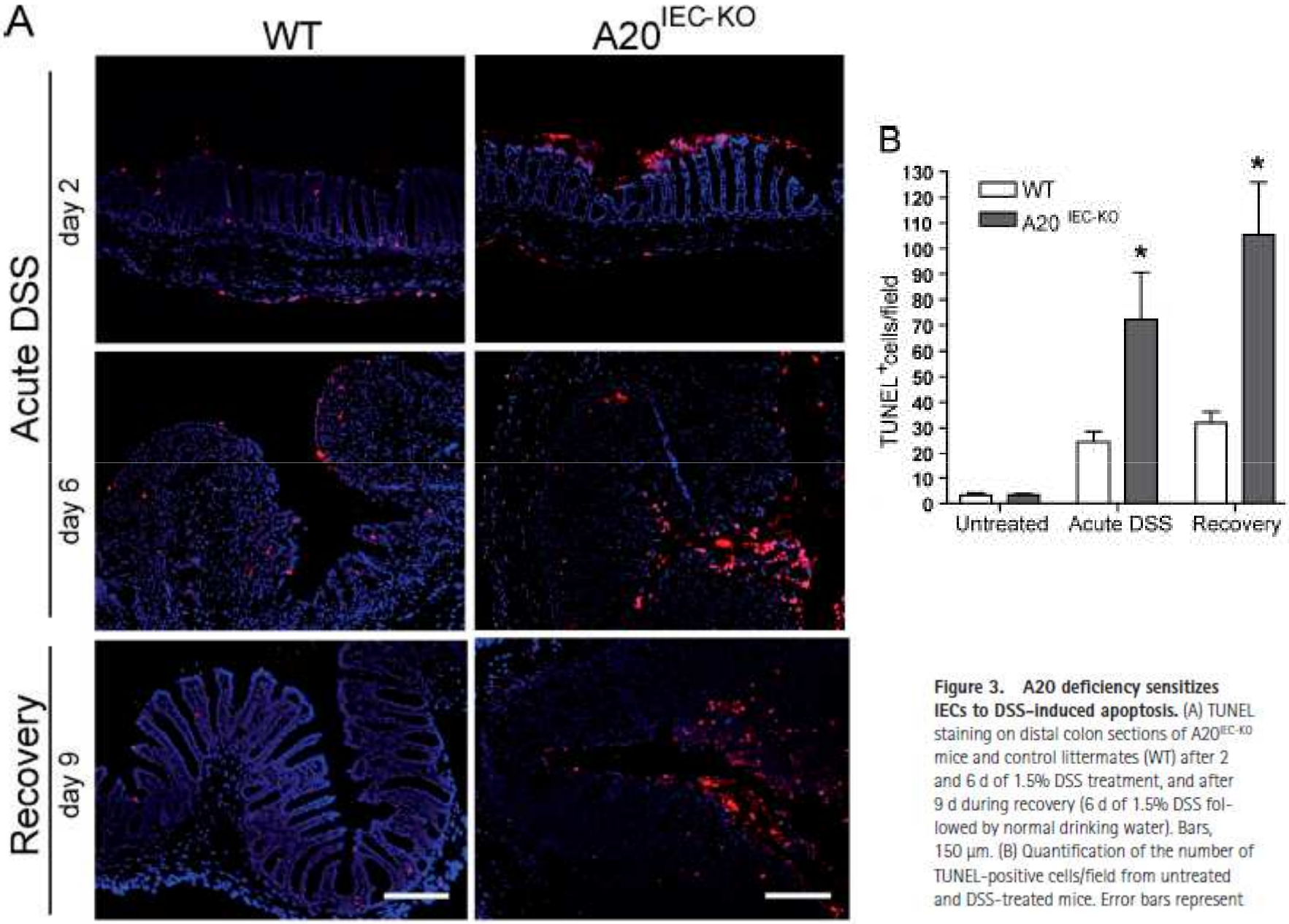
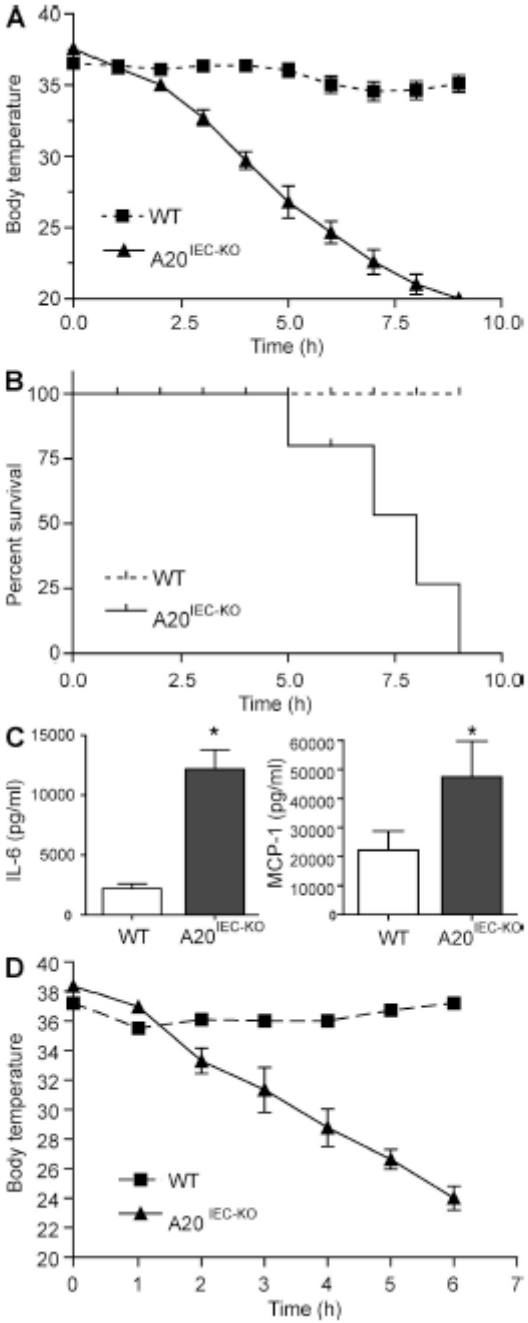


Figure 3. A20 deficiency sensitizes IECs to DSS-induced apoptosis. (A) TUNEL staining on distal colon sections of $A20^{IEC-KO}$ mice and control littermates (WT) after 2 and 6 d of 1.5% DSS treatment, and after 9 d during recovery (6 d of 1.5% DSS followed by normal drinking water). Bars, 150 µm. (B) Quantification of the number of TUNEL-positive cells/field from untreated and DSS-treated mice. Error bars represent



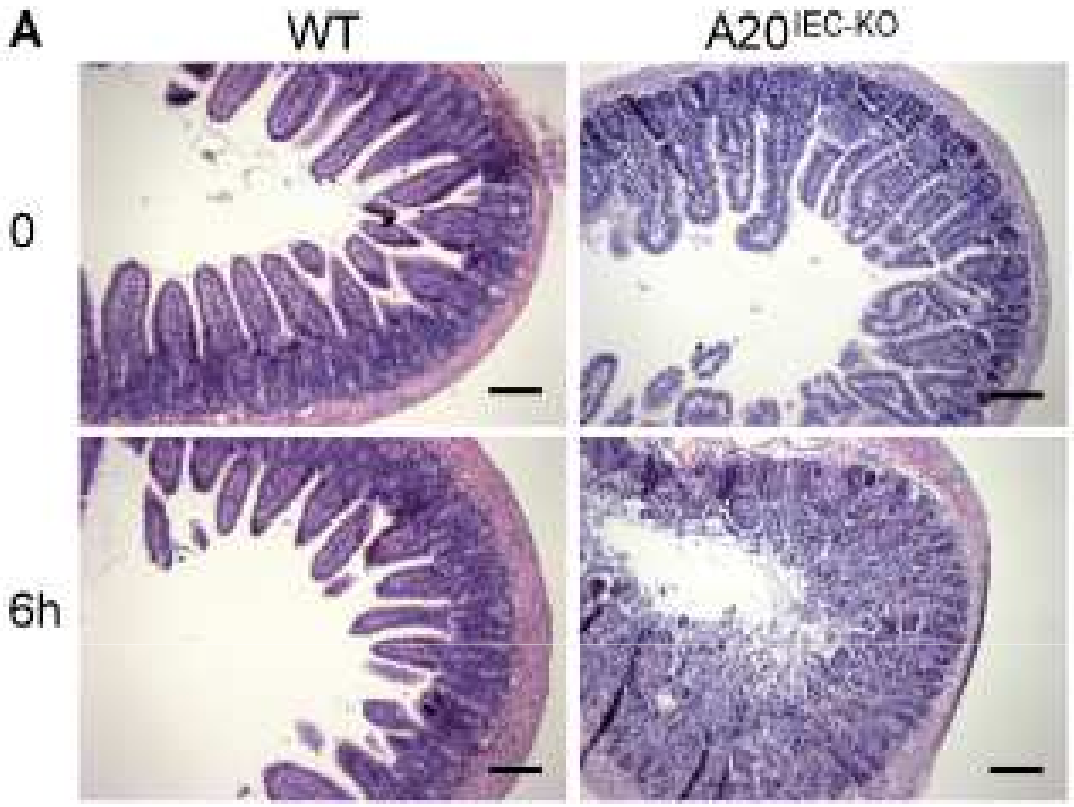


Figure 5. A20 deficiency in IECs sensitizes mice to TNF-induced toxicity. (A and B) Mice were injected i.p. with 5 µg of recombinant mouse TNF. Body temperature (A) and survival (B) of $A20^{IEC\text{-}KO}$ mice ($n = 8$) and littermate control mice (WT; $n = 6$). (C) Serum IL-6 and MCP-1 levels 4 h after mouse TNF injection. (D) Body temperature of $A20^{IEC\text{-}KO}$ mice ($n = 6$) and control littermate mice ($n = 6$) after injection with 50 µg of recombinant human TNF. Data are representative of three independent experiments. Error bars represent SEM. *, $P < 0.05$.

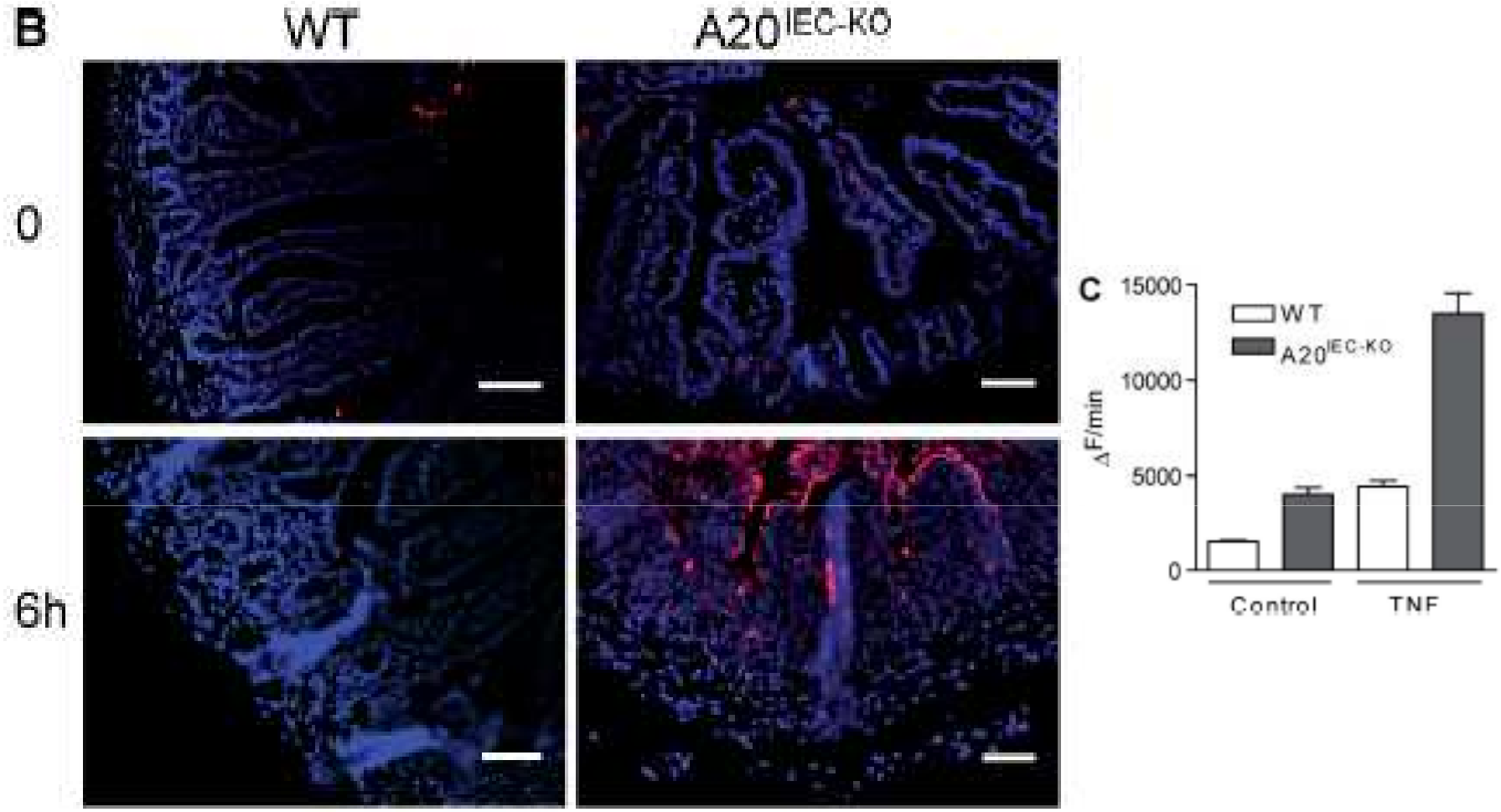


Figure 6. A20 deficiency in IECs sensitizes to TNF-induced damage of the small intestine and liver. (A) H&E histology on a section of the small intestine from $A20^{IEC-KO}$ mice and control littermates (WT) 0 and 6 h after mouse TNF injection. Bars, 100 µm. (B) TUNEL staining on sections from small intestine 0 and 6 h after mouse TNF injection, staining apoptotic cells in red. Bars, 50 µm. (C) Caspase activity assayed on tissue homogenates of terminal ileum of $A20^{IEC-KO}$ mice and WT littermates at 0 (control) and 90 min after mouse TNF injection. Error bars represent SEM. (D) H&E histology on liver sam-

Genetics

More recently, the search for IBD-associated genes has used genome-wide association studies (GWAS) that assess single-nucleotide polymorphisms. The number of genes identified by GWAS is increasing rapidly (already numbering more than 30), but along with **NOD2**, two Crohn disease–related genes of particular interest are **ATG16L1 (autophagy-related 16-like)**, a part of the autophagosome pathway that is critical to host cell responses to intracellular bacteria and, perhaps, epithelial homeostasis, and **IRGM (immunity-related GTPase M)**, which is also involved in autophagy and clearance of intracellular bacteria. NOD2, ATG16L1, and IRGM are expressed in multiple cell types, and their precise roles in Crohn disease pathogenesis have yet to be defined. However, like NOD2, ATG16L1 and IRGM are related to recognition and response to intracellular pathogens, supporting the hypothesis that inappropriate immune reactions to luminal bacteria are an important component of IBD pathogenesis. None of these genes are associated with ulcerative colitis. However, some polymorphisms of the IL-23 receptor are protective in both Crohn disease and ulcerative colitis.

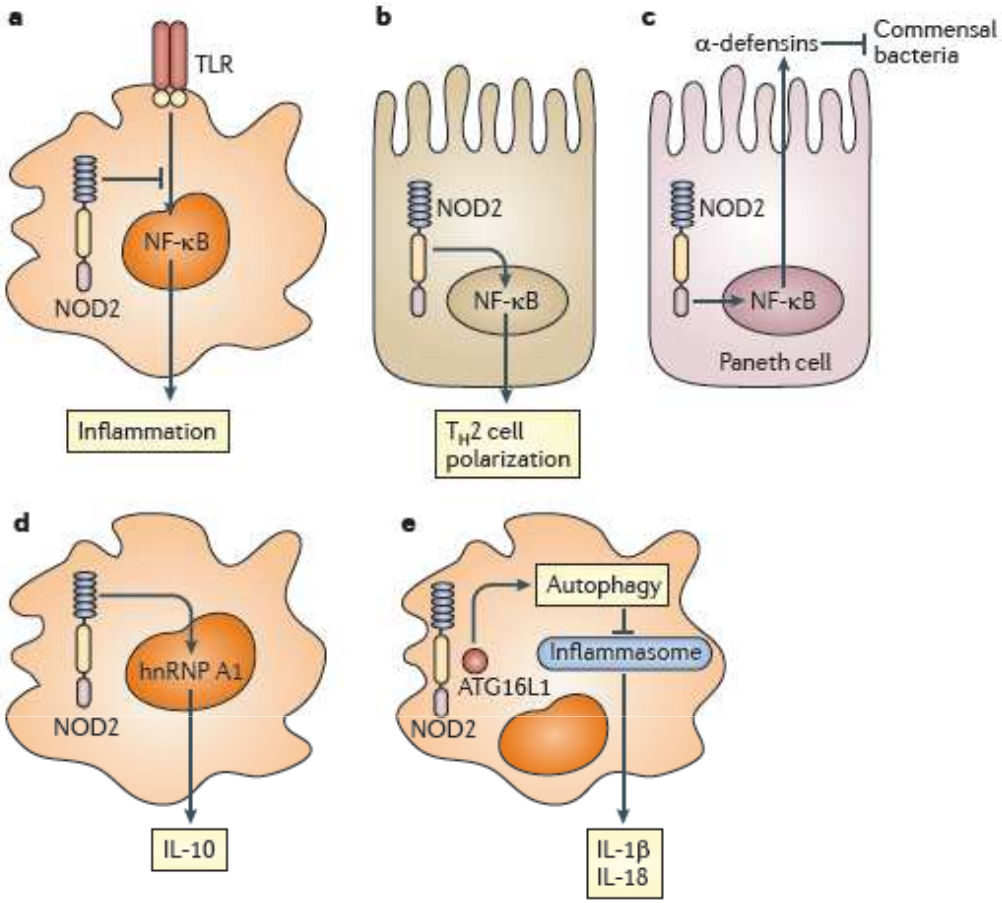
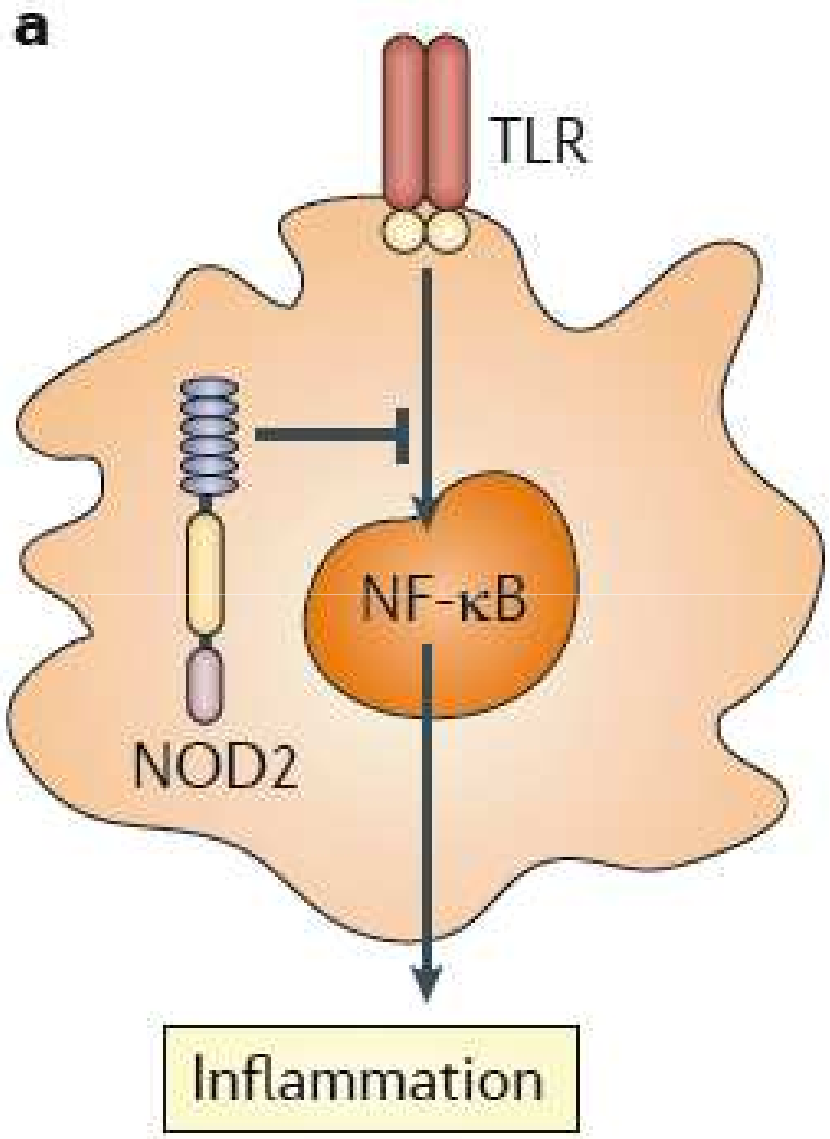


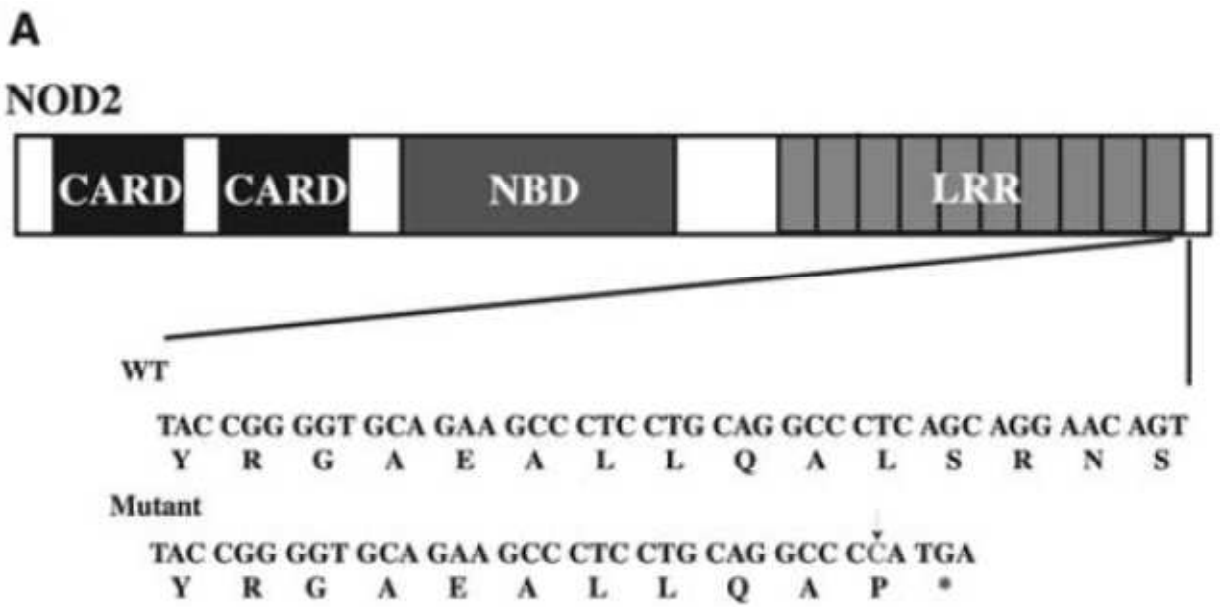
Figure 2 | **Proposed mechanisms of NOD2 function in intestinal homeostasis.** In addition to being expressed by ileal intestinal epithelial cells (IECs) and colonocytes, nucleotide-binding oligomerization domain 2 (NOD2) is predominantly expressed by myeloid cells such as macrophages and dendritic cells. Five different models have been described to account for the role of NOD2 in suppressing the inflammatory response in the gut. The first proposes that NOD2 inhibits Toll-like receptor (TLR) signalling (**a**). The second describes a role of NOD2 in skewing the T helper (T_H) cell response towards T_H2 cells (**b**). The third implicates NOD2 in α-defensin production and subsequent limitation of commensal bacterial numbers and microbiome composition (**c**). The fourth argues that human NOD2 stimulates the production of the anti-inflammatory cytokine interleukin-10 (IL-10) by regulating heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) (**d**) and that mutant NOD2 inhibits this process. Finally, the fifth model conjectures that NOD2 stimulates autophagy by interacting with autophagy-related 16-like 1 (ATG16L1), which inhibits the inflammasome thereby suppressing the production of the pro-inflammatory cytokines IL-1β and IL-18 (**e**). NF-κB, nuclear factor-κB.


a
TLR
NF-κB
NOD2
Inflammation

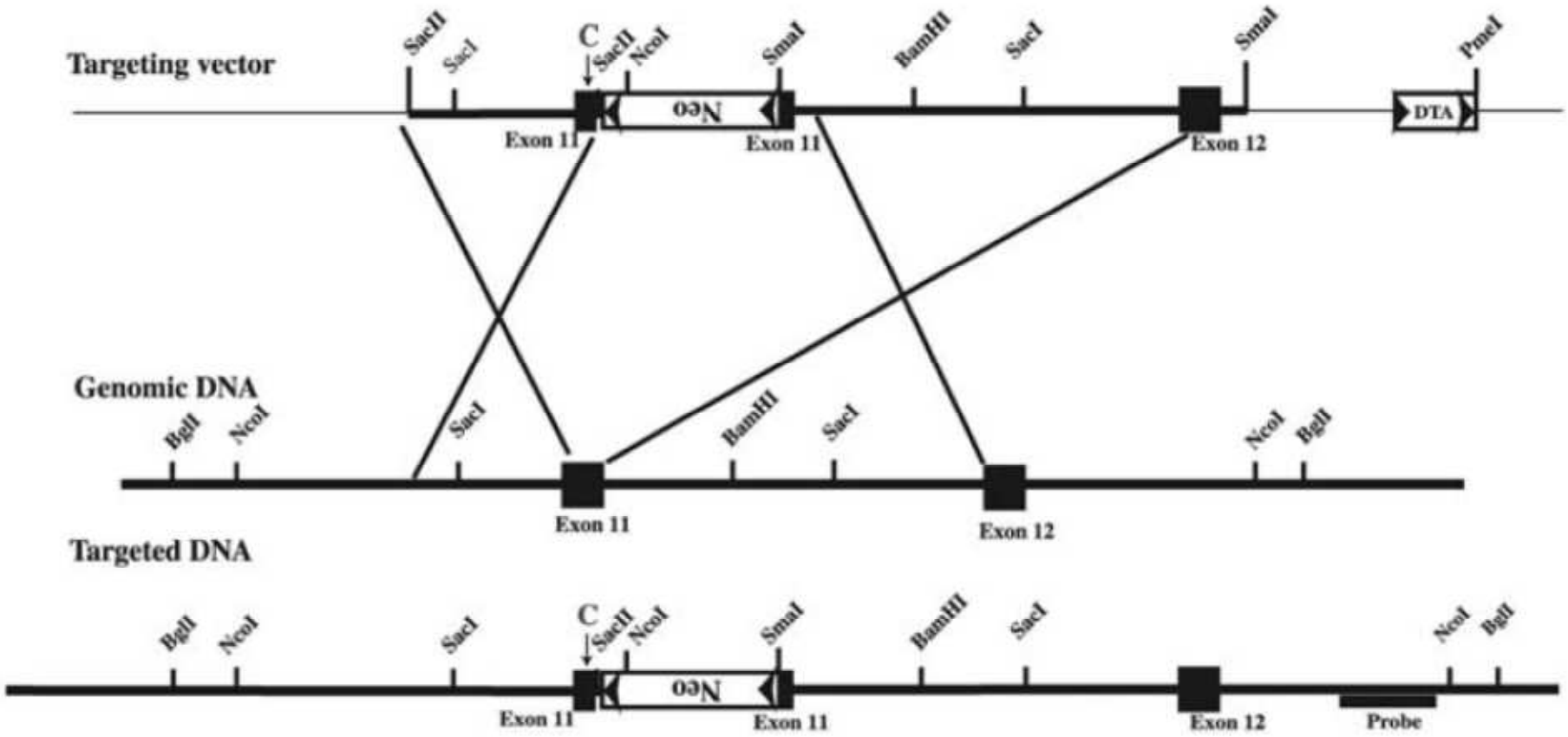
Nod2 Mutation in Crohn's Disease Potentiates NF-κB Activity and IL-1β Processing

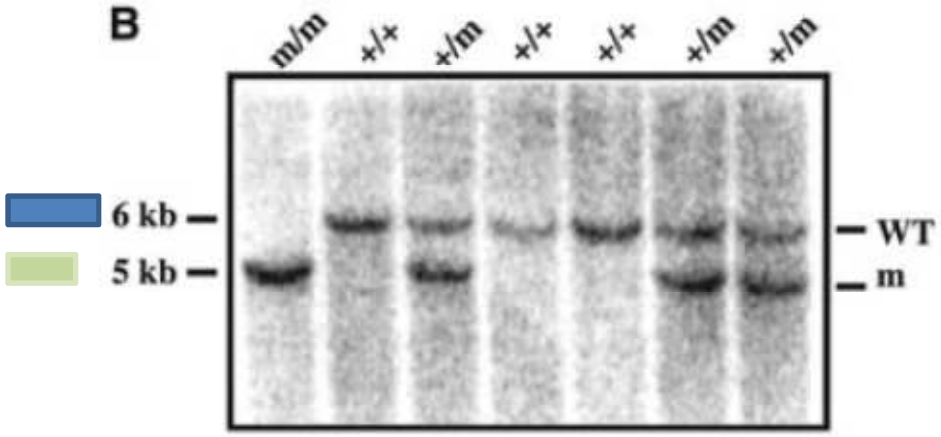
Shin Maeda,[1] Li-Chung Hsu,[1*] Hongjun Liu,[1*]
Laurie A. Bankston,[1,3] Mitsutoshi Iimura,[2] Martin F. Kagnoff,[2]
Lars Eckmann,[2] Michael Karin[1†]

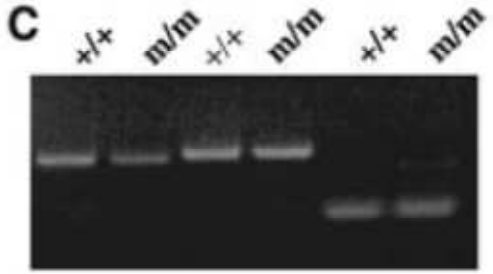
Variants of NOD2, an intracellular sensor of bacteria-derived muramyl dipeptide (MDP), increase susceptibility to Crohn's disease (CD). These variants are thought to be defective in activation of nuclear factor κB (NF-κB) and antibacterial defenses, but CD clinical specimens display elevated NF-κB activity. To illuminate the pathophysiological function of NOD2, we introduced such a variant to the mouse *Nod2* locus. Mutant mice exhibited elevated NF-κB activation in response to MDP and more efficient processing and secretion of the cytokine interleukin-1β (IL-1β). These effects are linked to increased susceptibility to bacterial-induced intestinal inflammation and identify NOD2 as a positive regulator of NF-κB activation and IL-1β secretion.

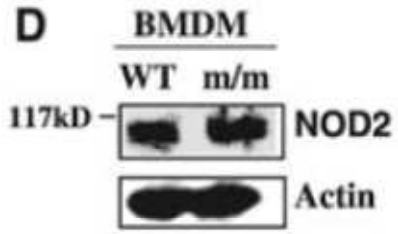


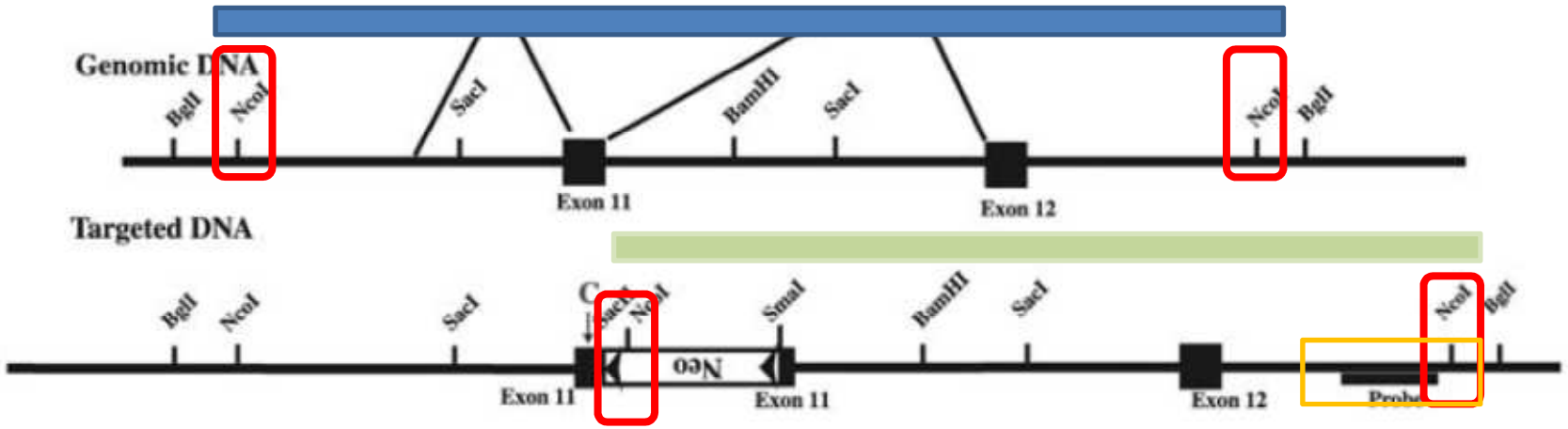
which CD-associated *NOD2* variants act, we generated mice whose *Nod2* locus harbors the homolog of the most common CD susceptibility allele, *3020insC*, which encodes a truncated protein lacking the last 33 amino acids (*3*, *4*). This was done through insertion of cytosine at position 2939 (corresponding to 3020 in human *NOD2*) of the *Nod2* open reading frame (Fig. 1, A and B). Homozygous $Nod2^{2939iC}$ mice were obtained at the expected mendelian ratio and did not show abnormalities of the gastrointestinal tract

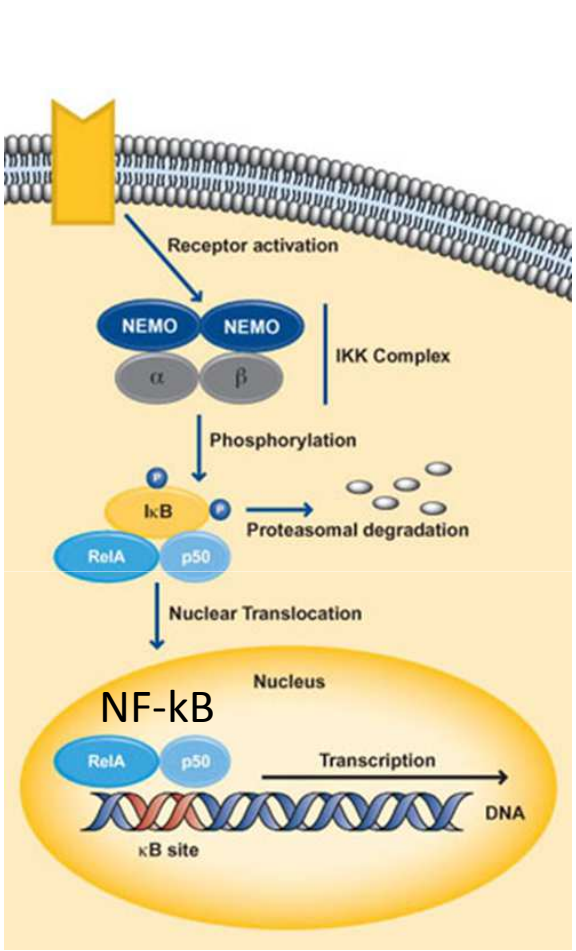

Targeting vector
SacII
SacI
C
SacII
NcoI
SmaI
BamHI
SacI
SmaI
PmeI
Neo
DTA
Exon 11
Exon 11
Exon 12
Genomic DNA
BglI
NcoI
SacI
BamHI
SacI
NcoI
BglI
Exon 11
Exon 12
Targeted DNA
BglI
NcoI
SacI
C
SacII
NcoI
SmaI
BamHI
SacI
NcoI
BglI
Neo
Exon 11
Exon 11
Exon 12
Probe


B
m/m
+/+
+/m
+/+
+/+
+/m
+/m
6 kb
5 kb
WT
m


C
+/+
m/m
+/+
m/m
+/+
m/m


D
BMDM
WT
m/m
117kD
NOD2
Actin


Genomic DNA
BglI
NcoI
SacI
BamHI
SacI
NcoI
BglI
Exon 11
Exon 12
Targeted DNA
BglI
NcoI
SacI
SmaI
BamHI
SacI
NcoI
BglI
Neo
Exon 11
Exon 11
Exon 12



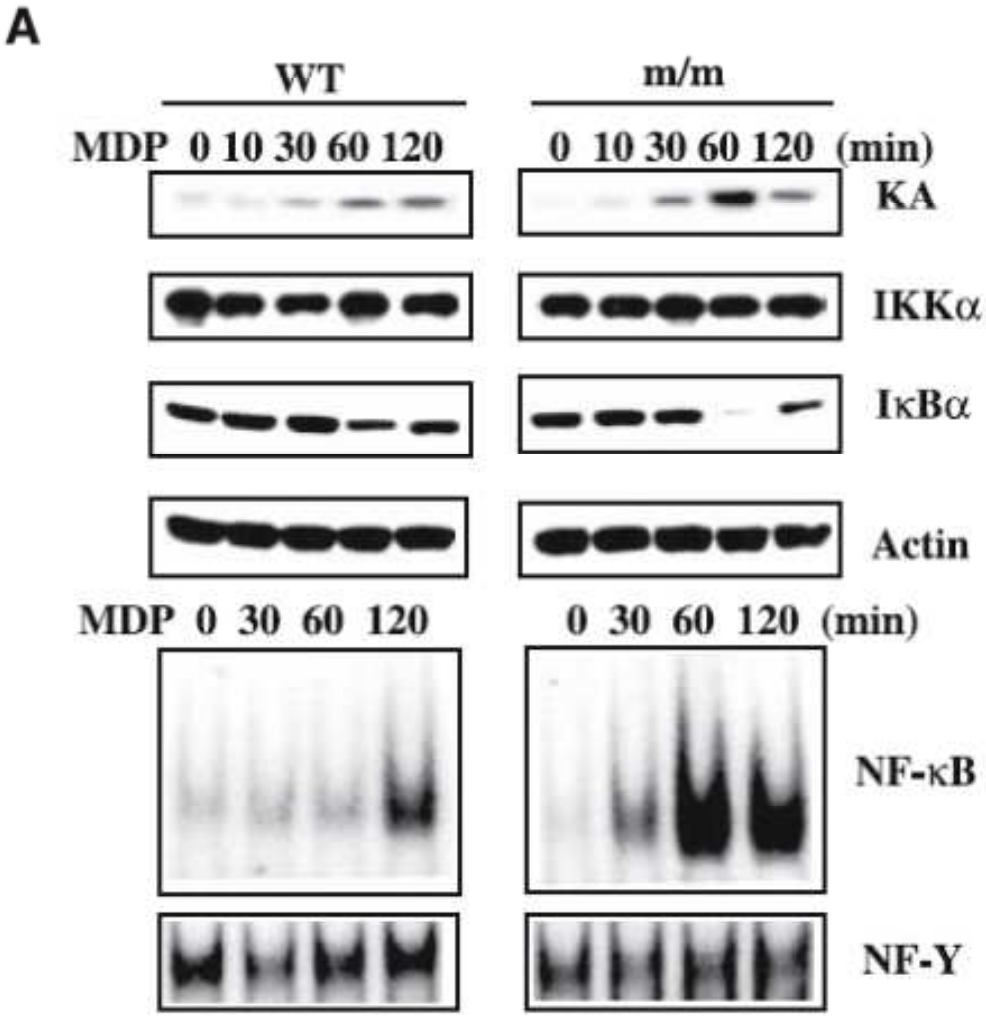
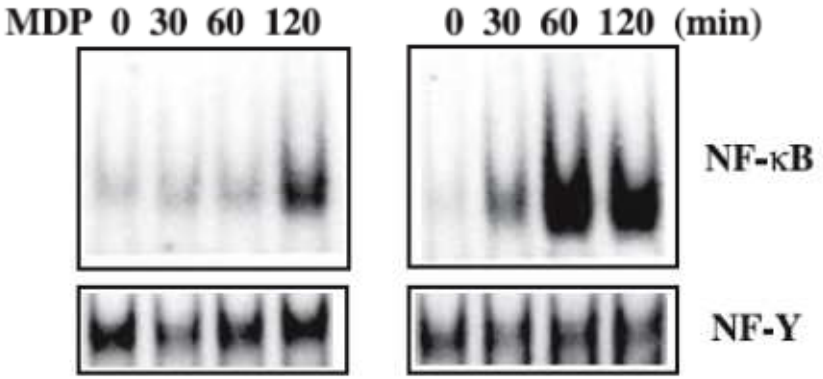
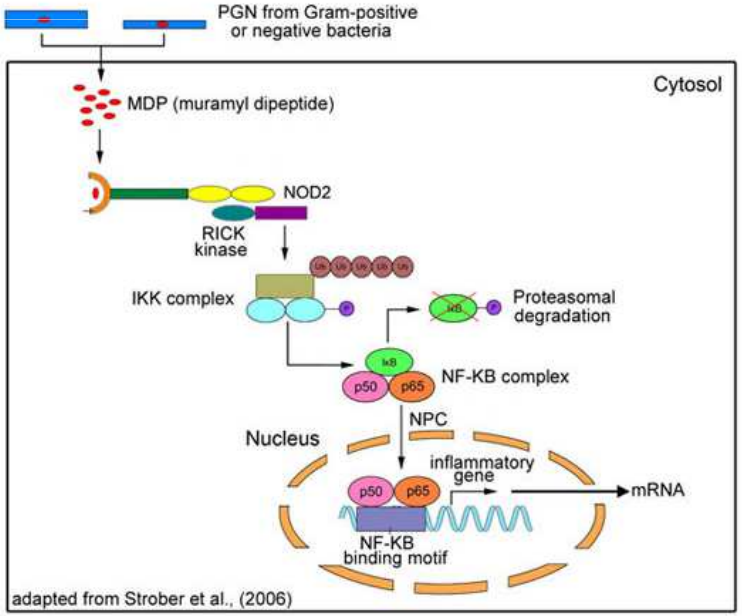
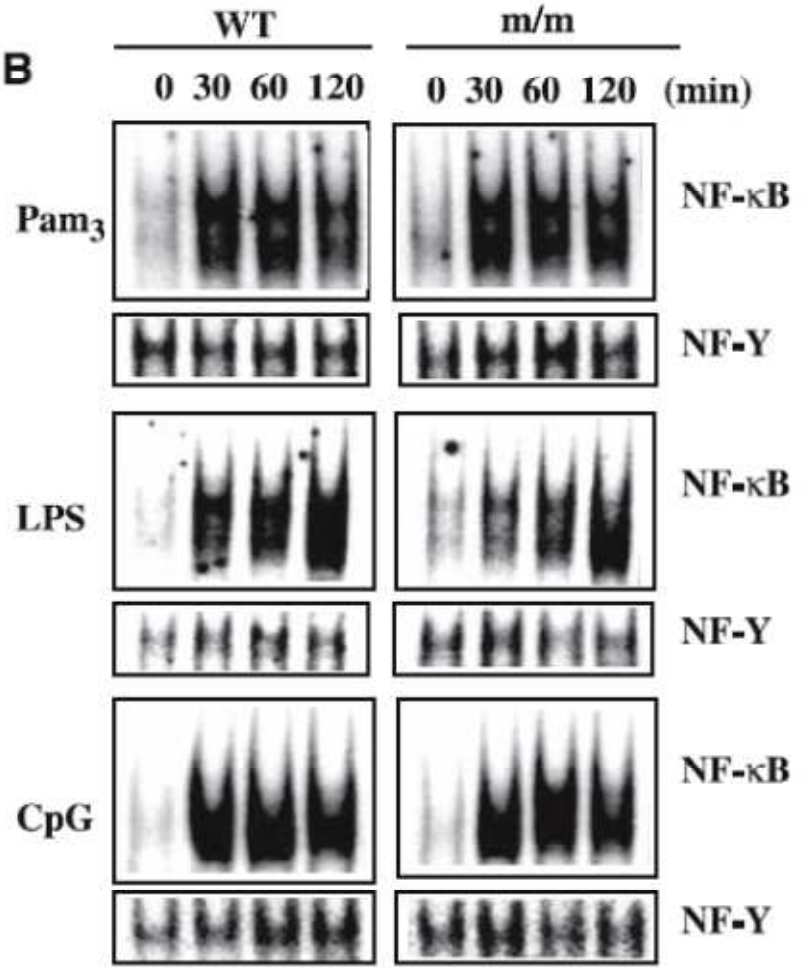
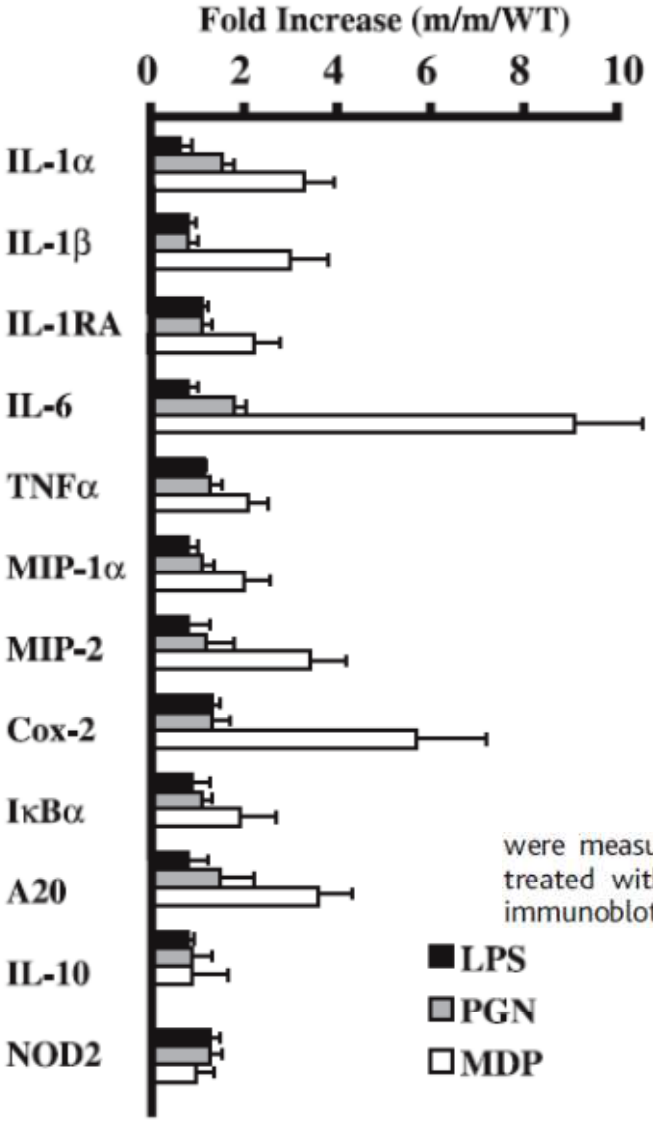


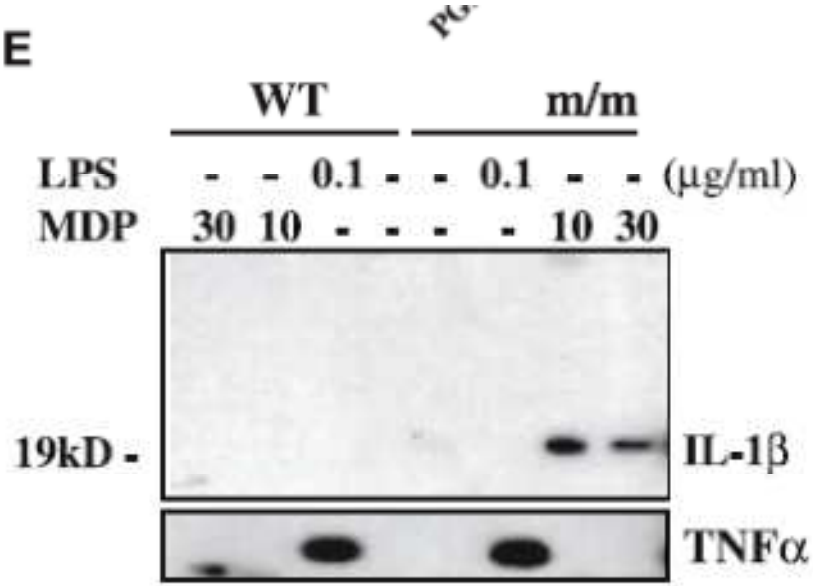
Fig. 2. *Nod2*^{2939iC} macrophages exhibit elevated NF-κB activation and IL-1β secretion in response to MDP. (**A**) BMDMs from WT and *Nod2*^{2939iC} (m/m) mice were incubated with MDP (1 μg/ml). Where indicated, cytosolic and nuclear extracts were prepared and used to analyze IKK activation (KA), IκBα degradation, and NF-κB DNA binding activity, respectively. Nuclear extract quality was monitored by measuring nuclear factor-Y (NF-Y) DNA binding. (**B**) BMDMs were stimulated with


MDP 0 30 60 120
0 30 60 120 (min)
NF-κB
NF-Y


PGN from Gram-positive
or negative bacteria
Cytosol
MDP (muramyl dipeptide)
NOD2
RICK
kinase
IKK complex
Proteasomal
degradation
IκB
p50
p65
NF-KB complex
NPC
Nucleus
inflammatory
gene
mRNA
NF-KB
binding motif
adapted from Strober et al., (2006)


B
WT
m/m
0 30 60 120
0 30 60 120 (min)
Pam3
LPS
CpG
NF-κB
NF-Y





were measured. (**E**) MDP induces IL-1β release by *Nod2*^{2939iC} (m/m) BMDMs. Macrophages were treated with MDP or LPS for 24 hours. Culture supernatants were collected and analyzed by immunoblotting with antibodies against IL-1β and TNFα.

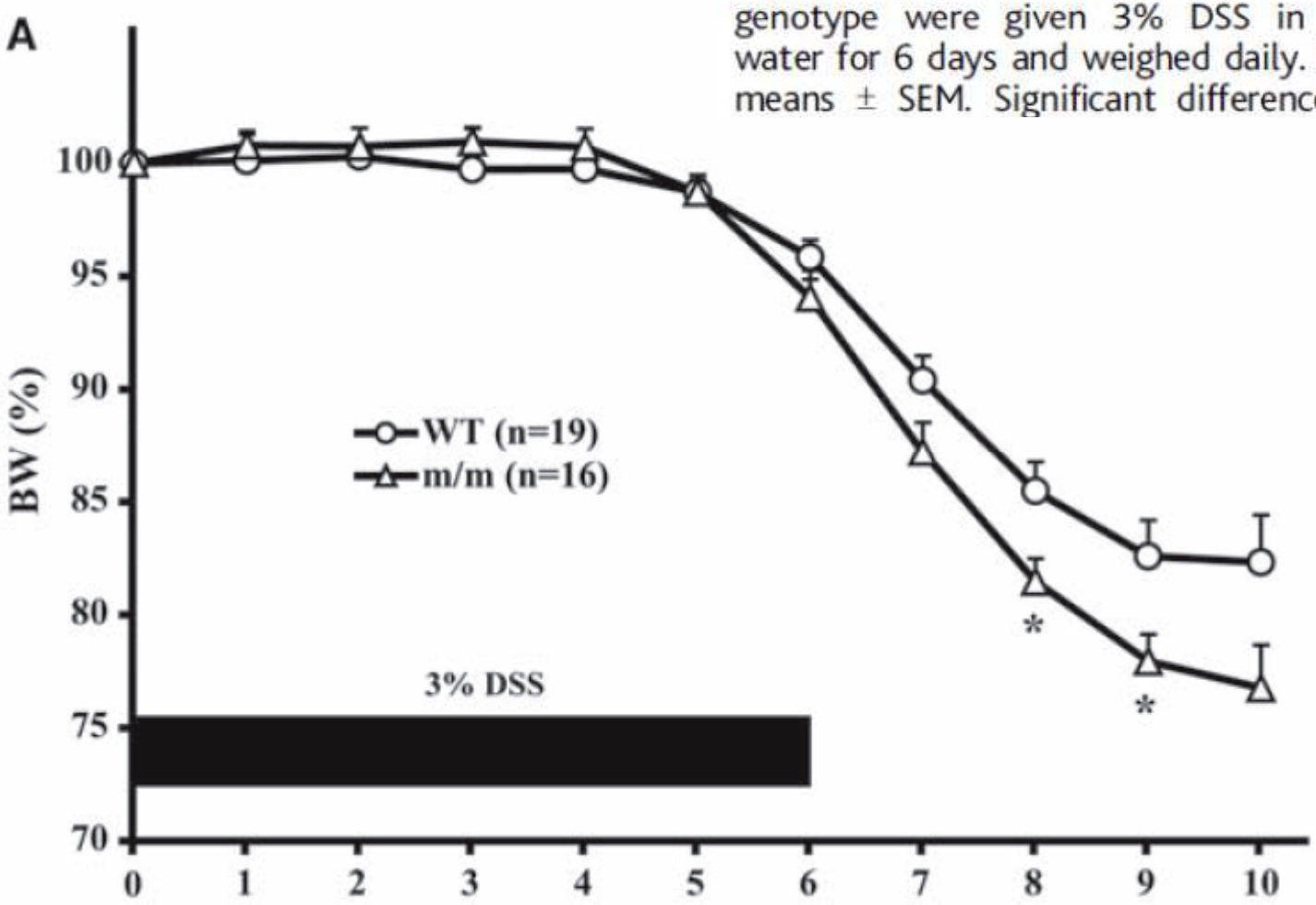
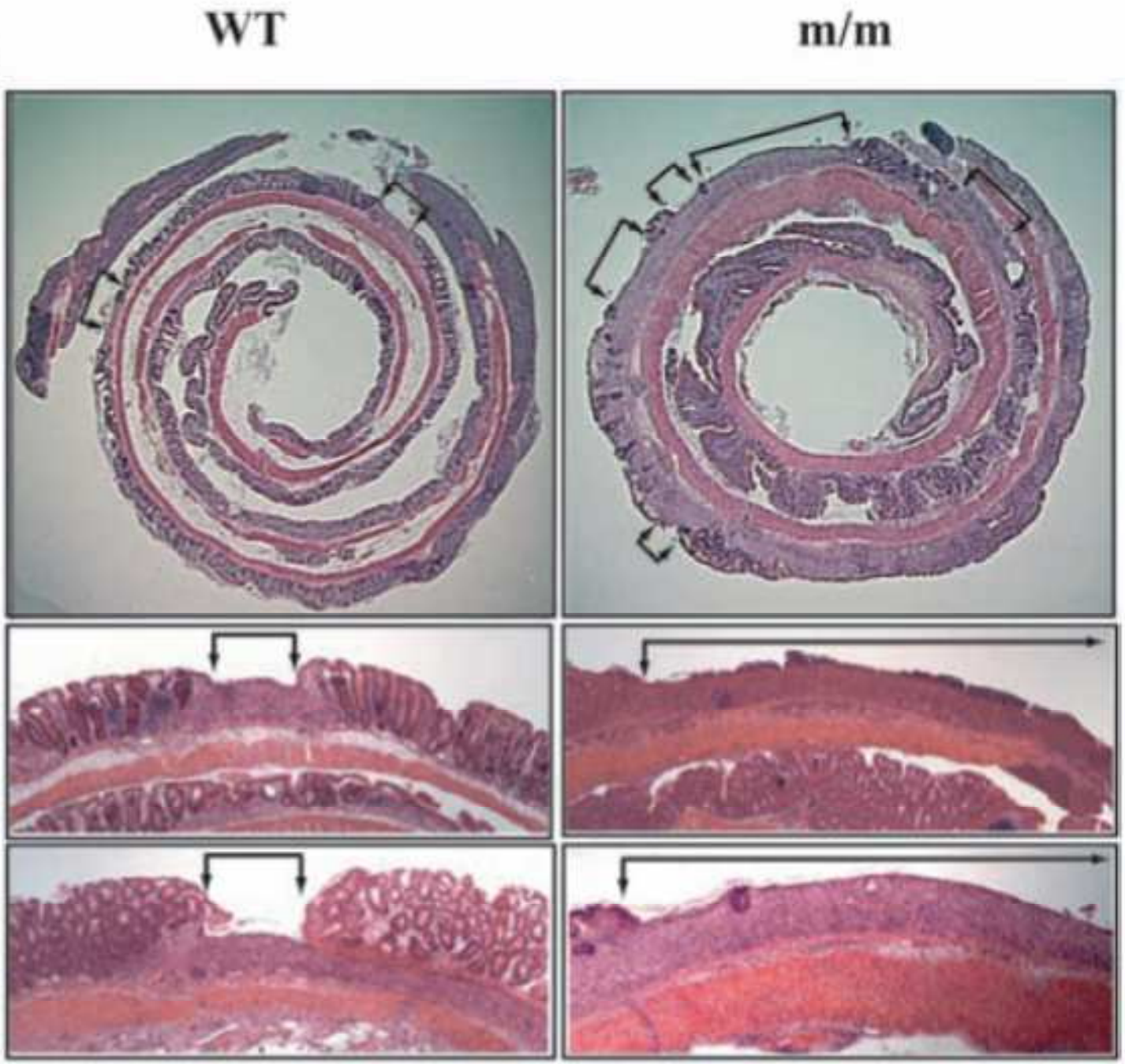
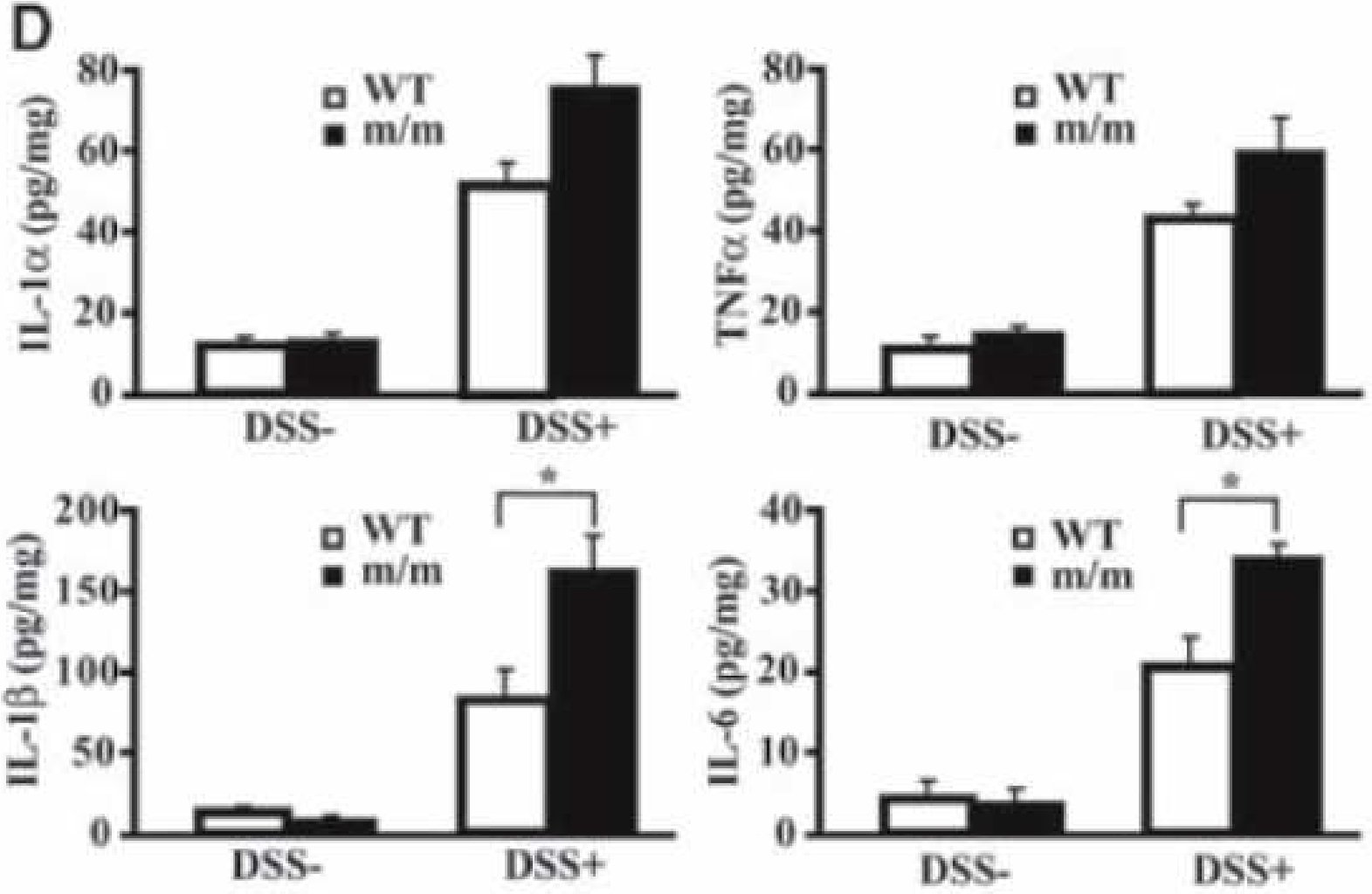
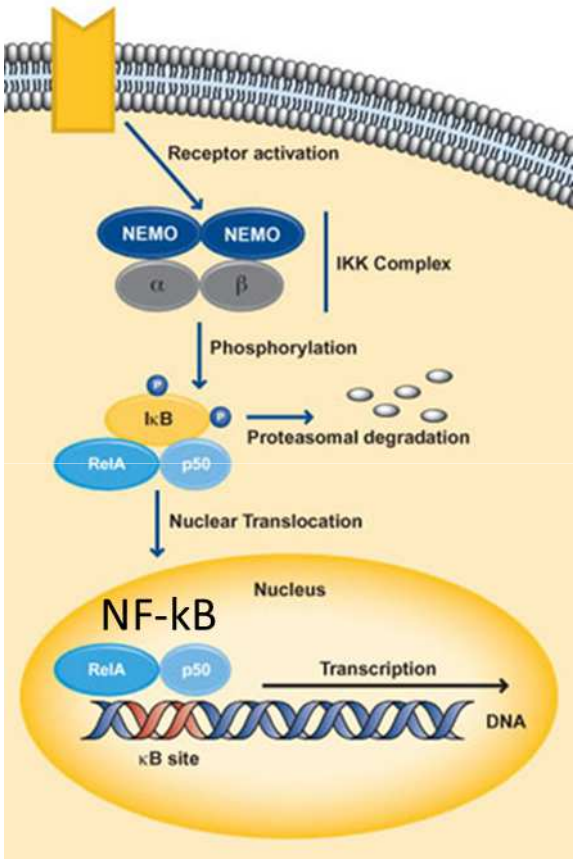


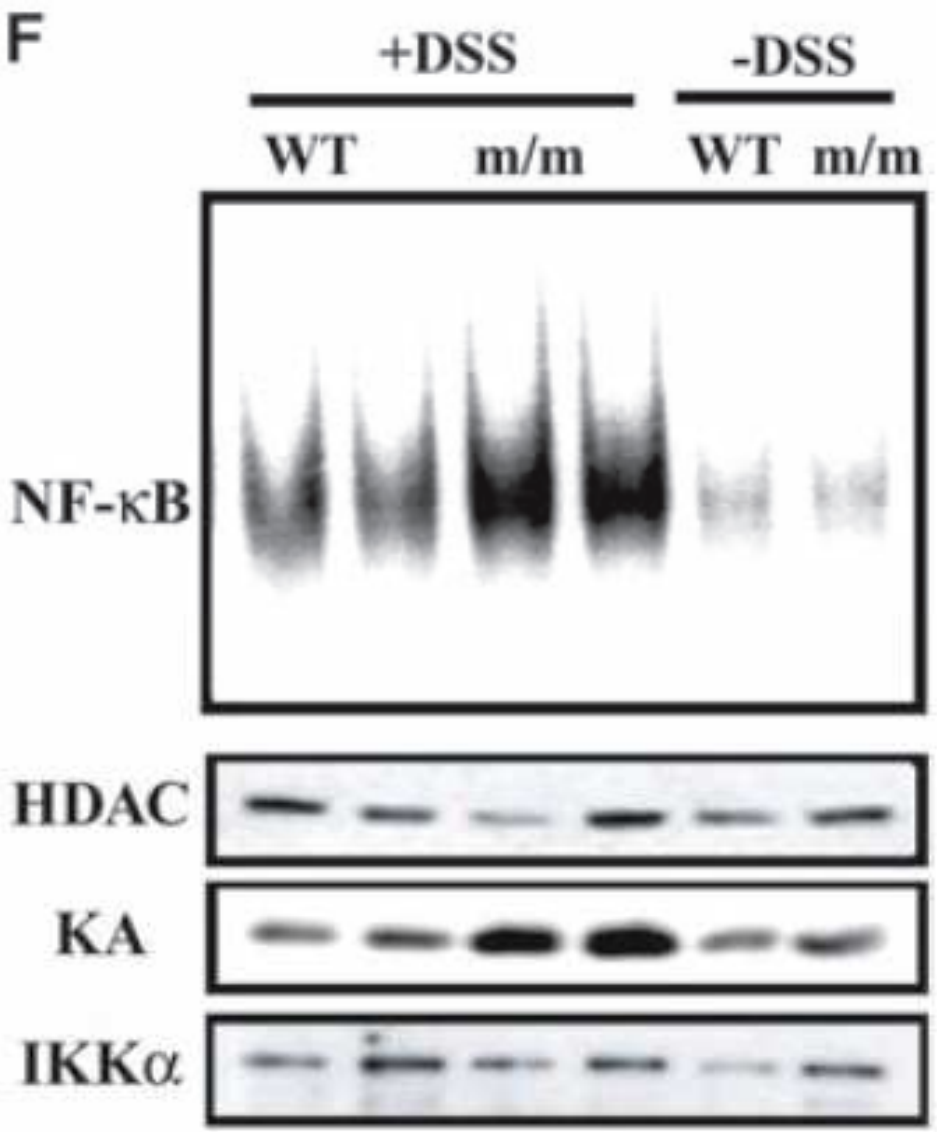
Fig. 3. Enhanced NF-κB activation and inflammation in DSS-treated *Nod2*^{2939iC} mice. (**A**) Increased body weight loss in DSS-exposed *Nod2*^{2939iC} mice. Mice of either genotype were given 3% DSS in drinking water for 6 days and weighed daily. Data are means ± SEM. Significant differences, *P <

B




D
IL-1α (pg/mg)
80
60
40
20
0
WT
m/m
DSS-
DSS+
TNFα (pg/mg)
80
60
40
20
0
WT
m/m
DSS-
DSS+
IL-1β (pg/mg)
200
150
100
50
0
*
WT
m/m
DSS-
DSS+
IL-6 (pg/mg)
40
30
20
10
0
*
WT
m/m
DSS-
DSS+


Receptor activation
NEMO
NEMO
IKK Complex
α
β
Phosphorylation
P
IκB
P
Proteasomal degradation
RelA
p50
Nuclear Translocation
Nucleus
NF-kB
RelA
p50
Transcription
DNA
κB site


F
+DSS
-DSS
WT
m/m
WT
m/m
NF-κB
HDAC
KA
IKKα

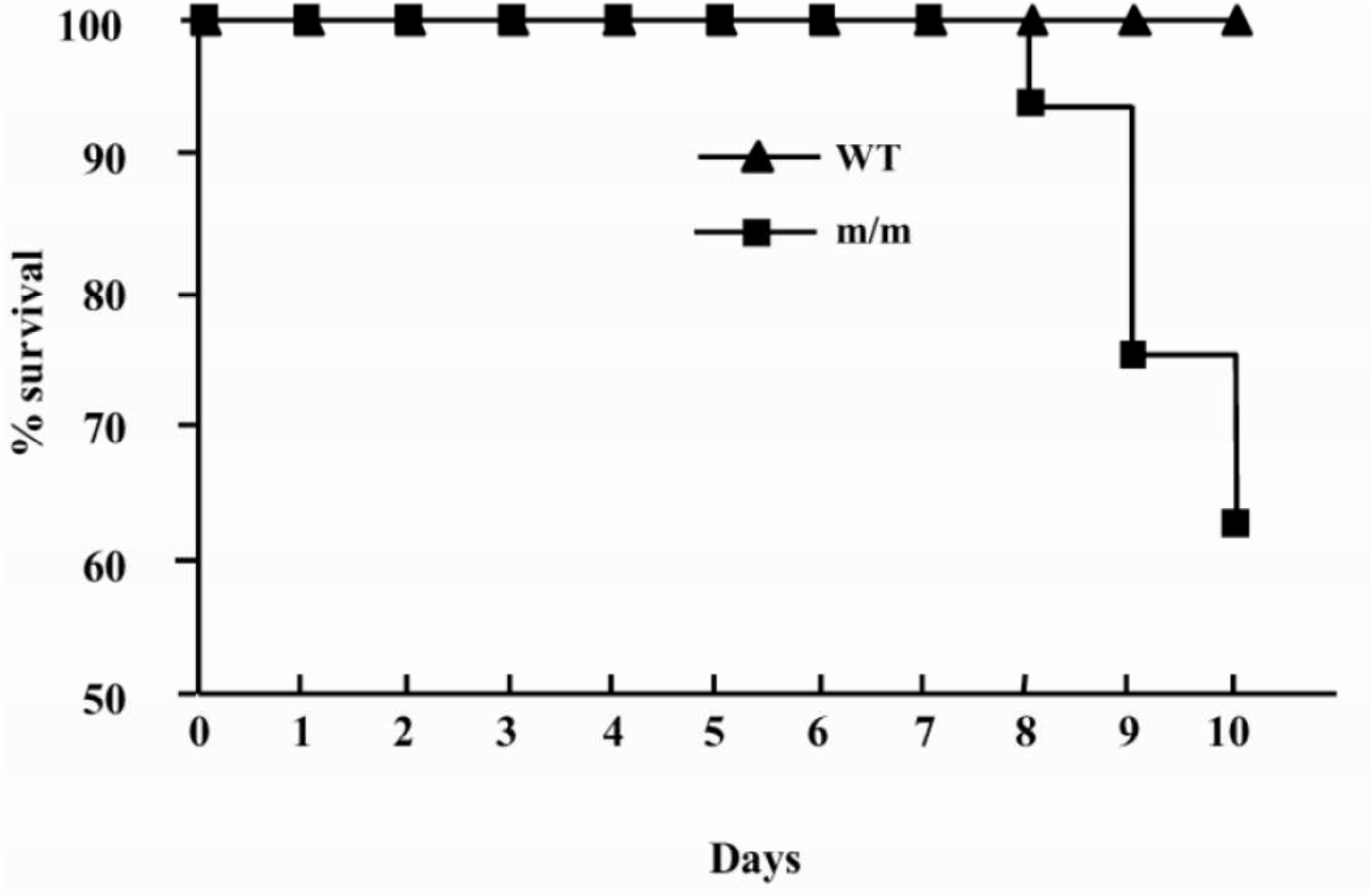
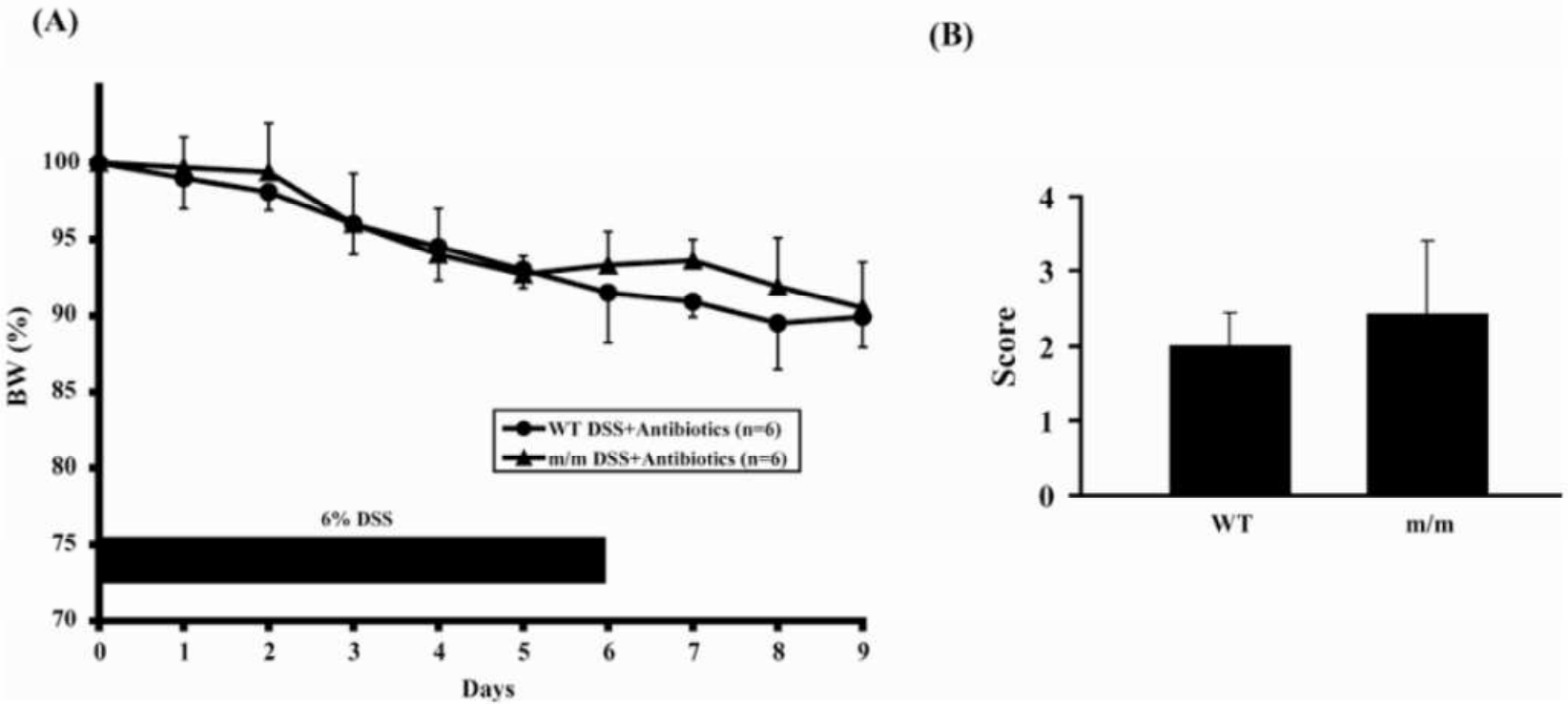
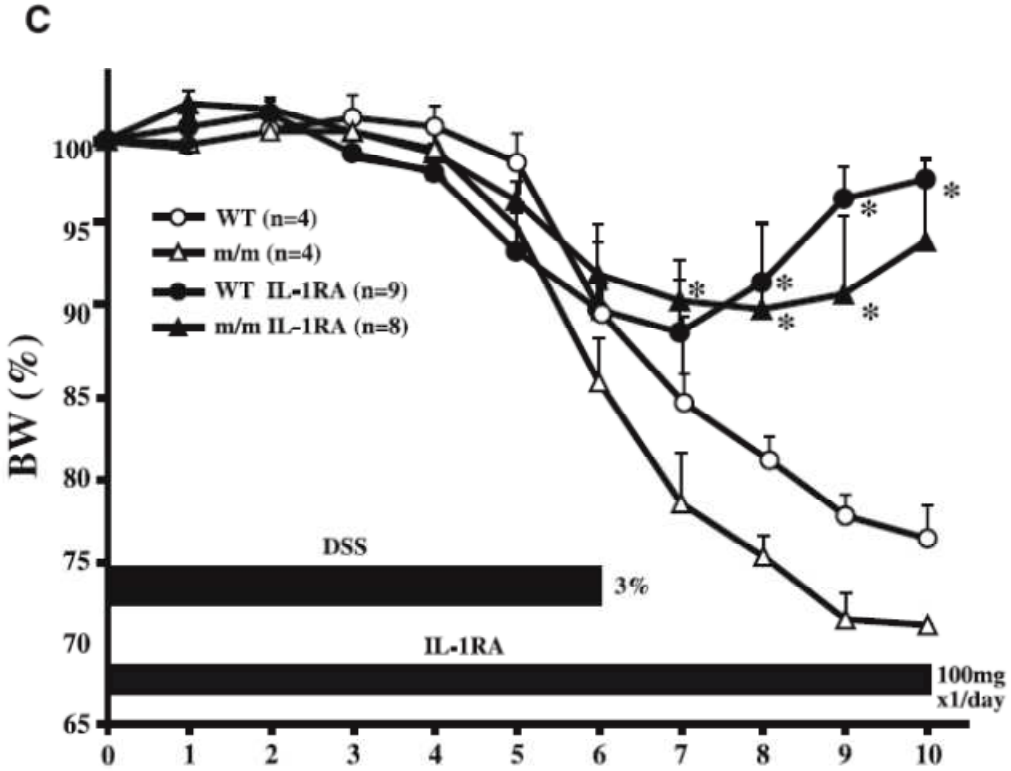
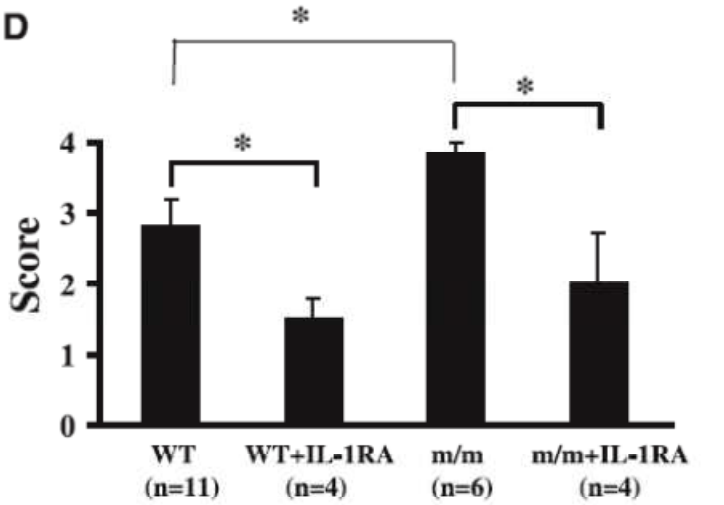


Fig. S4. Survival curves of WT (n = 19) and *Nod2*^{2939iC} (n = 16) mice treated with DSS (3%) for 6 days. Significantly increased mortality was found in *Nod2*^{2939iC} mice relative to WT mice (37.5% vs. 0%) by 10 days after DSS exposure.

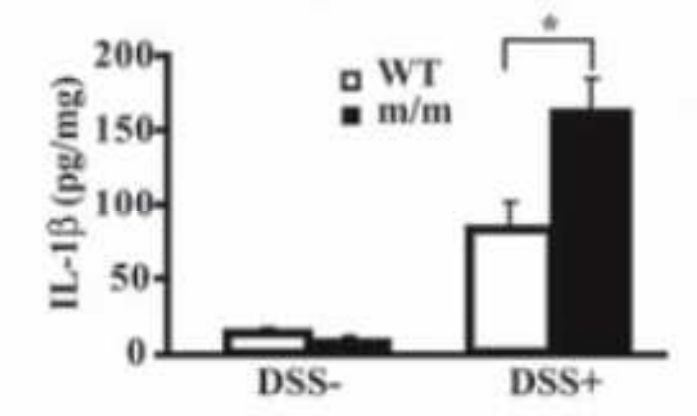

(A)
100
95
90
85
80
75
70
BW (%)
WT DSS+Antibiotics (n=6)
m/m DSS+Antibiotics (n=6)
6% DSS
0
1
2
3
4
5
6
7
8
9
Days
(B)
Score
4
3
2
1
0
WT
m/m

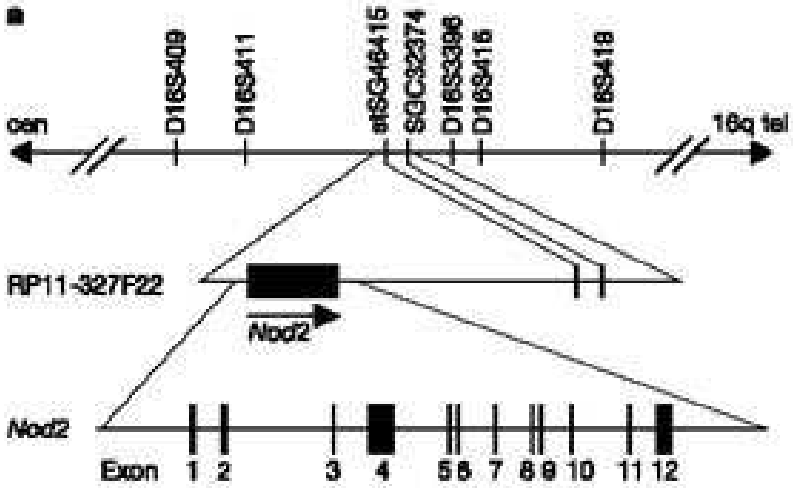


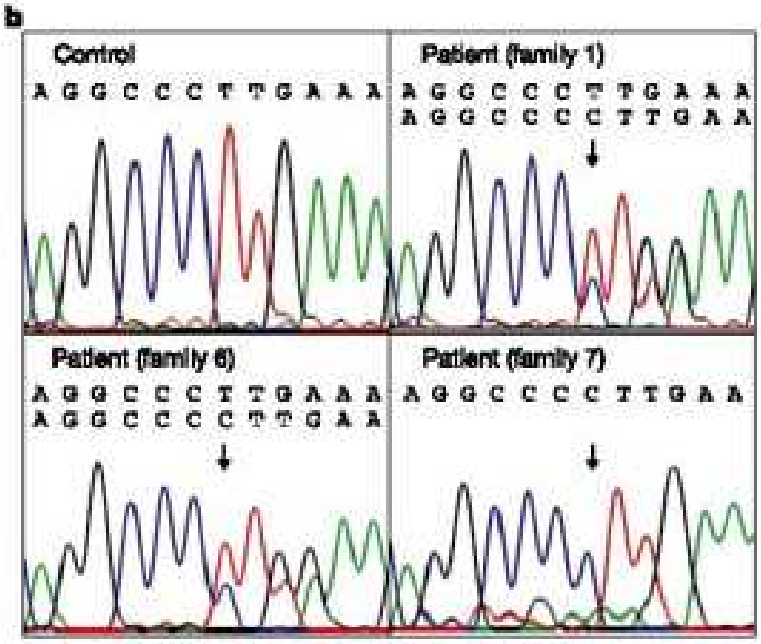


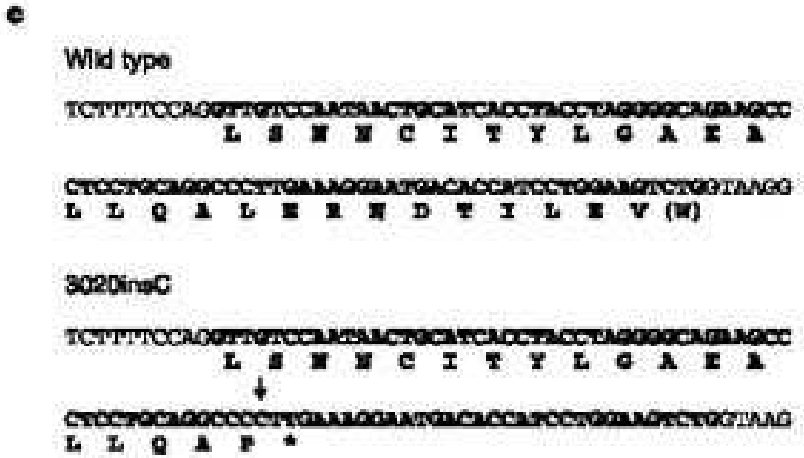
initiation of DSS treatment in the mice from (C). Significant differences, *$P < 0.05$.

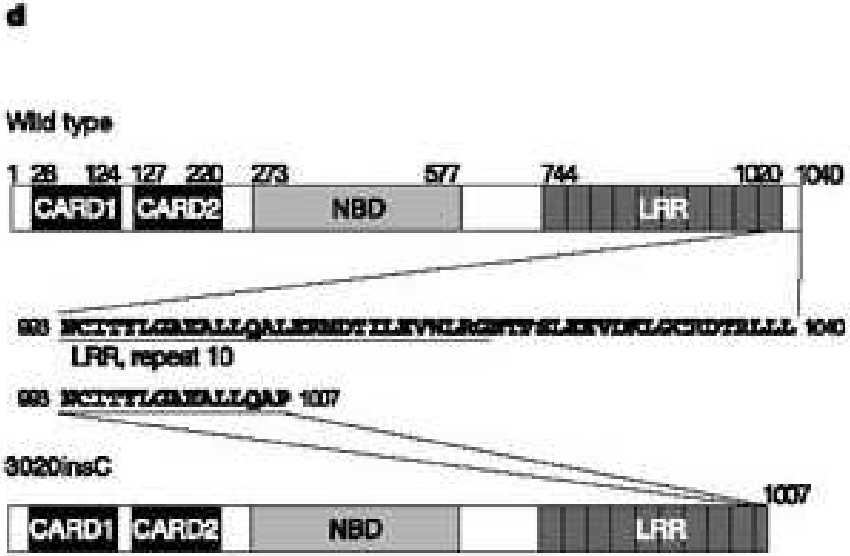
(C) Increased body weight loss in DSS-exposed Nod22939iC mice is IL-1β dependent. Mice of either genotype were given 3% DSS for 6 days with or without concomitant treatment with IL-1RA (100 mg/kg per day). Mice were weighed daily. Data are means ± SEM. Asterisks indicate significant differences (WT versus WT IL-1-RA, m/m versus m/m IL-1-RA; $P < 0.05$). (D) Histological inflammation and tissue damage scores were determined 11 days after initiation of DSS treatment in the mice from (C). Results are means ± SEM. Significant differences, *$P < 0.05$.




a
cen
D16S409
D16S411
rs6446415
SGC32374
D16S3396
D16S416
D16S418
16q tel
RP11-327F22
Nod2
Nod2
Exon 1 2 3 4 5 6 7 8 9 10 11 12

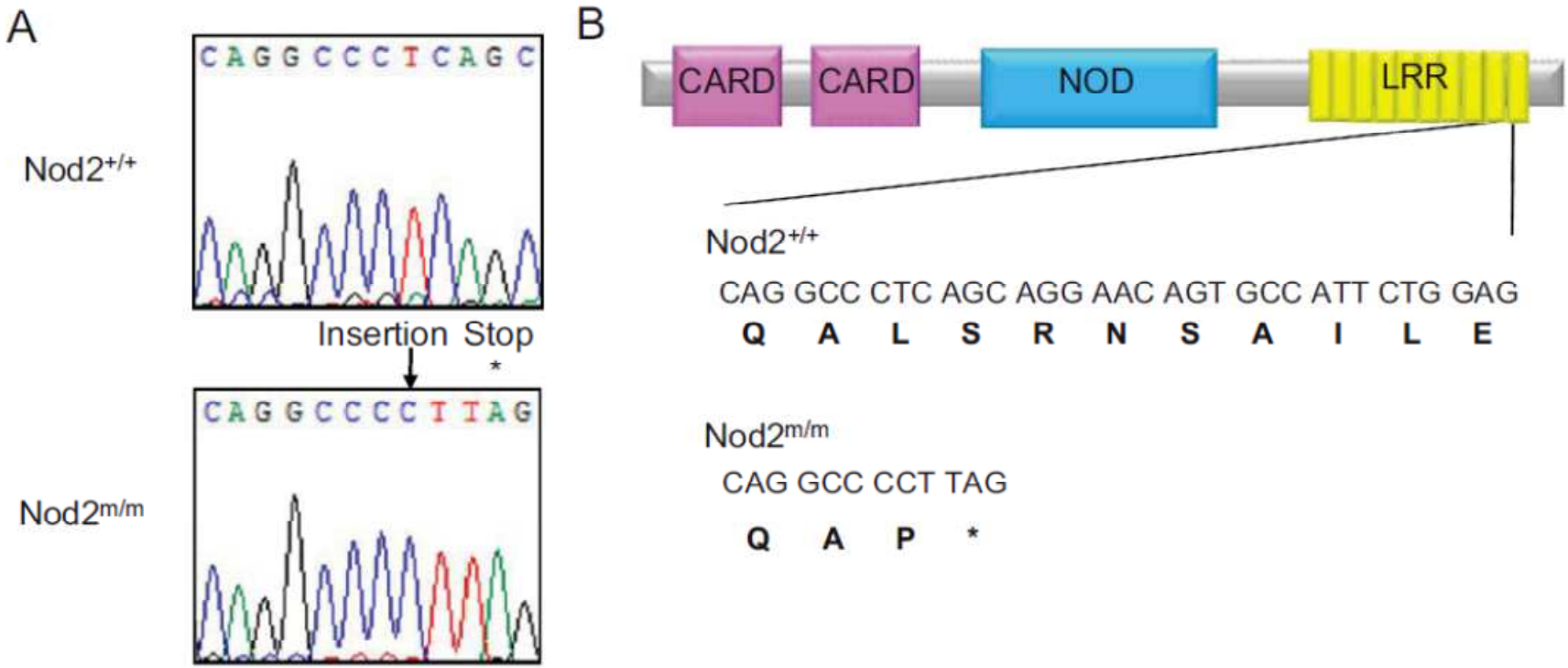

b
Control
A G G C C C T T G A A A
Patient (family 1)
A G G C C C T T G A A A
A G G C C C C T T G A A
Patient (family 6)
A G G C C C T T G A A A
A G G C C C C T T G A A
Patient (family 7)
A G G C C C C T T G A A

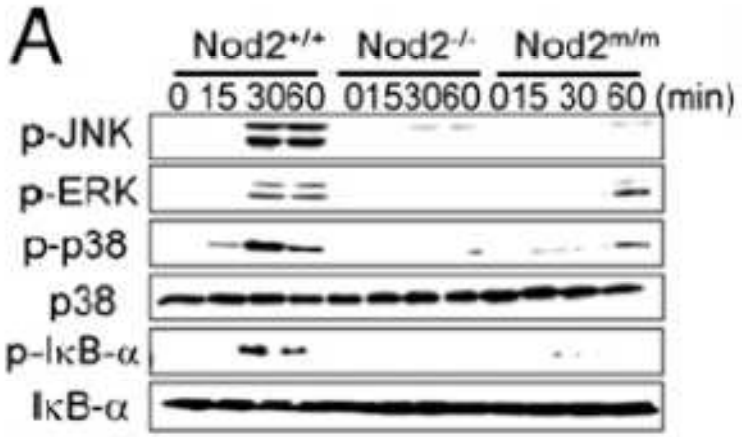

c
Wild type
L S N N C I T Y L G A K A
L L Q A L E K N D T I L E V (W)
3020insC
L S N N C I T Y L G A K A
L L Q A F *

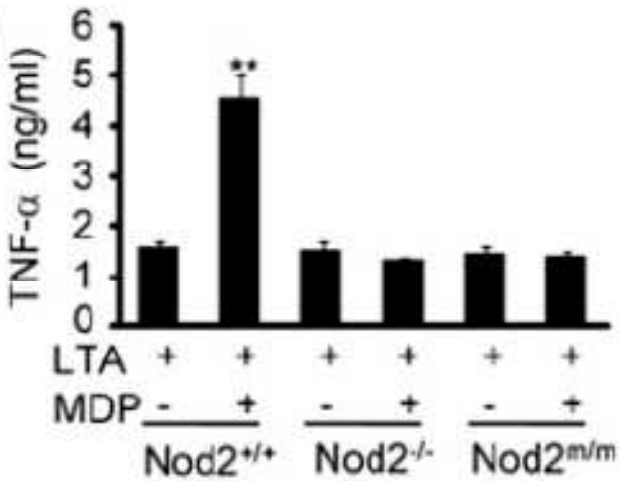

d
Wild type
1 26 124 127 220 273 577 744 1020 1040
CARD1
CARD2
NBD
LRR
LRR, repeat 10
3020insC
1007
CARD1
CARD2
NBD
LRR

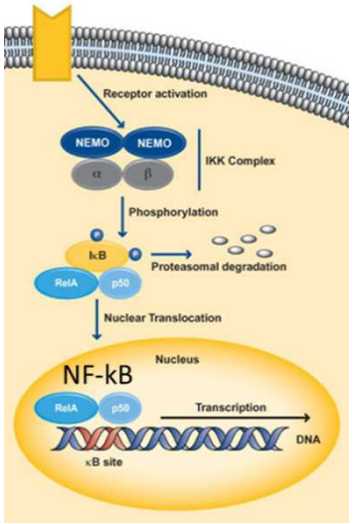
Cutting Edge: Crohn's Disease-Associated Nod2 Mutation Limits Production of Proinflammatory Cytokines To Protect the Host from *Enterococcus faecalis*-Induced Lethality

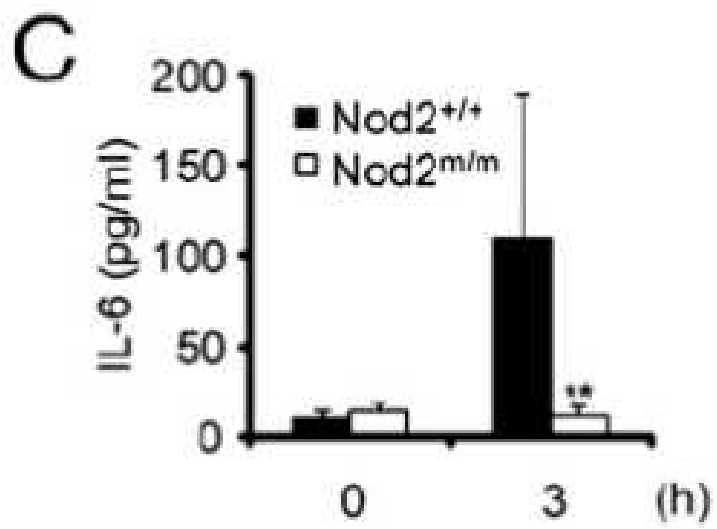
Yun-Gi Kim, Michael H. Shaw, Neil Warner, Jong-Hwan Park,[1] Felicia Chen, Yasunori Ogura,[2] and Gabriel Núñez

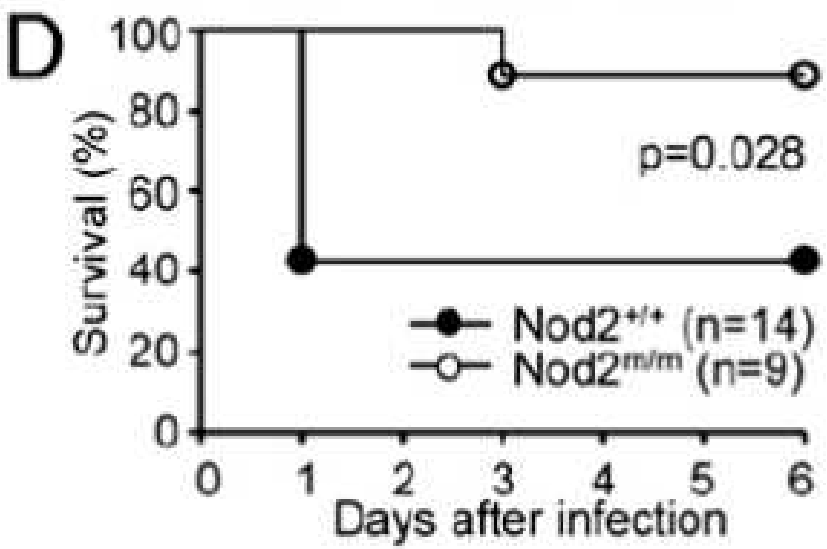



A
Nod2+/+
Nod2-/-
Nod2m/m
0 15 3060 0153060 015 30 60 (min)
p-JNK
p-ERK
p-p38
p38
p-IκB-α
IκB-α

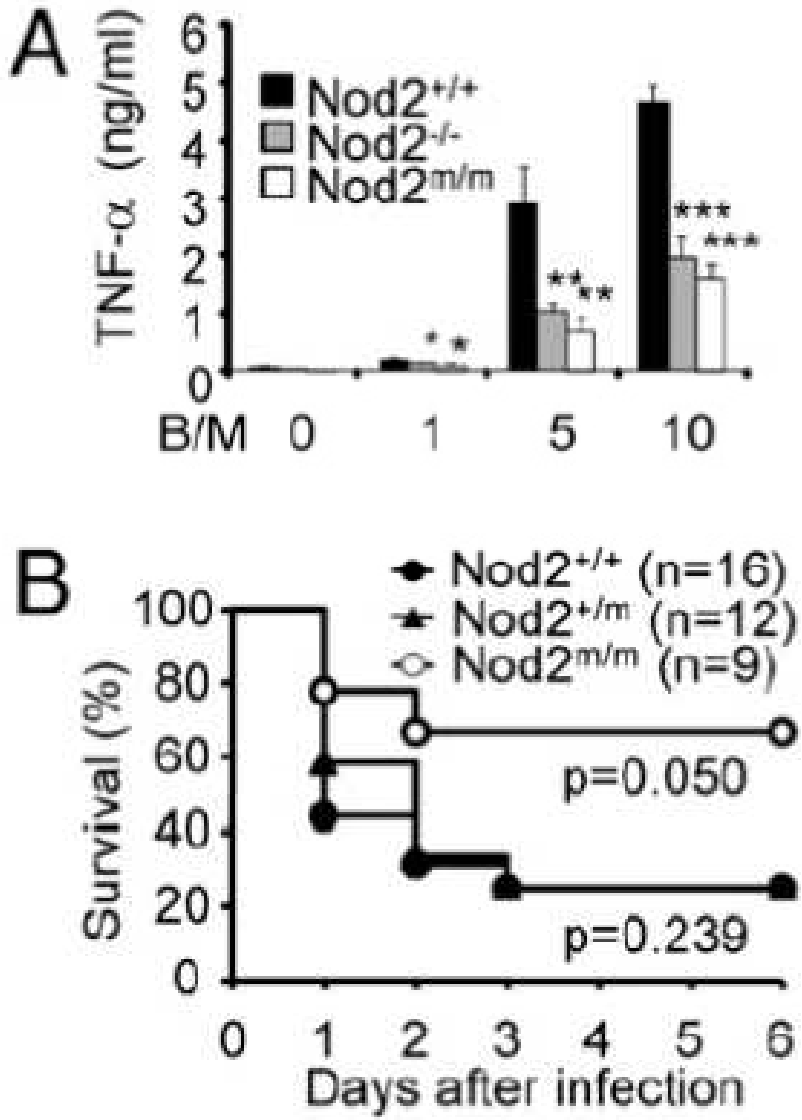

B
TNF-α (ng/ml)
6
5
4
3
2
1
0
**
LTA + + + + + +
MDP - + - + - +
Nod2+/+
Nod2-/-
Nod2m/m


Receptor activation
NEMO
NEMO
α
β
IKK Complex
Phosphorylation
IκB
Proteasomal degradation
RelA
p50
Nuclear Translocation
Nucleus
NF-kB
RelA
p50
Transcription
DNA
κB site

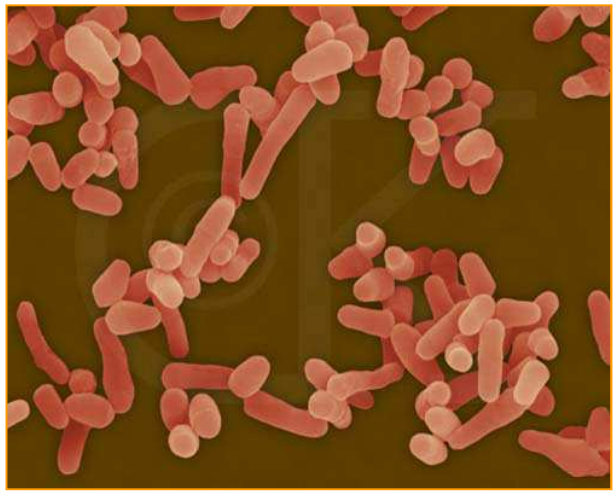




Enterococcus faecalis


A
TNF-α (ng/ml)
6
5
4
3
2
1
0
Nod2+/+
Nod2-/-
Nod2m/m
*
**
**
***
***
B/M 0 1 5 10
B
Survival (%)
100
80
60
40
20
0
Nod2+/+ (n=16)
Nod2+/m (n=12)
Nod2m/m (n=9)
p=0.050
p=0.239
0 1 2 3 4 5 6
Days after infection

Un modello spontaneo di IBD: Paratubercolosi-Johne’s disease



Mycobacterium avium paratuberculosis

• L'infezione di *Mycobacterium johnei* in bovino è responsabile di una patologia con notevoli analogie rispetto al morbo di Crohn.

• Potrebbe essere un infezione da micobatteri la causa scatenante il morbo di Crohn?

Table 1

A comparison of Crohn's disease in man with chronic mycobacterial enteritis of ruminants

	Crohn's disease	*Johne's disease*
Early confusion with intestinal tuberculosis	Yes	Yes
Incidence	Familial	Breed susceptibility (e.g. Channel Island breeds)
Chronic diarrhœa and wasting	Yes	Yes
Acute episodes induced by 'stress'	No	Yes
'Regional ileitis' but other sites may be affected	Yes	Yes
Adaptation – subject may be clinically healthy when gut is severely affected	Yes	Yes
'Cobblestone' appearance of gut due to submucosal thickening	Yes	Yes
Ulceration and fistula formation	Yes	Not seen
Caseation necrosis	No	Occasionally in sheep, not in cattle
Aggregation of epithelioid cells into granulomatous lesions	Yes	Yes
Etiology	?Hypersensitivity state caused by unknown infective or sensitizing agent	Hypersensitivity reaction to specific infection by *Myco. johnei*

TABLE 1
Evidence supporting and not supporting *Mycobacterium avium* subspecies *paratuberculosis* (MAP) as the etiological agent in Crohn's disease (CD)

The evidence supporting MAP as a cause of CD

1. The similarity between Johne's Disease and CD (4)
2. MAP has been found in milk and water supplies and is capable of surviving commercial pasteurization methods (23,24)
3. MAP has been detected in the tissues and blood of CD patients with a greater frequency than those without CD (15,26,27,43)
4. Positive antibodies to MAP antigens in the blood of CD patients compared with controls (17,44,45)
5. Detection of MAP in human breast milk from patients with CD (29)
6. The gene *NOD2/CARD15* has previously been shown to be a susceptibility gene for the development of CD (11,31). *NOD2/CARD15* mutations result in a defective innate response to bacterial infection and, possibly, ineffective clearance of intracellular MAP

The evidence not supporting MAP as a cause of CD

1. Humans exposed to animals infected with MAP do not show a higher prevalence of CD (22)
2. MAP has been isolated from individuals without CD, albeit in smaller numbers (13,28). This would suggest that MAP is at least, not a sufficient cause for CD and that other factors are necessary to induce disease
3. There is a lack of evidence that consumption of food containing MAP organisms causes CD (25)
4. There is no evidence to support increased transmission of MAP and CD to offspring despite the report of MAP cultured from breast milk of MAP-infected mothers with CD (30)
5. CD responds to immunosuppressive therapy, such as corticosteroids, which has been associated with decreased levels of MAP DNA (14). *Mycobacterium tuberculosis* proliferates with antitumour necrosis factor-alpha antibodies or corticosteroid treatment and *Mycobacterium intracellulare* flourishes as CD4 counts fall with acquired immunosuppression, yet similar results have not been found in MAP infection (30,35)
6. A randomized controlled trial (37) of antibiotics active against MAP for two years with a one-year follow-up period failed to show any sustained benefit in the treatment of CD beyond an initial response to treatment in the first 16 weeks

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Article

Interleukin-10-deficient mice develop chronic enterocolitis

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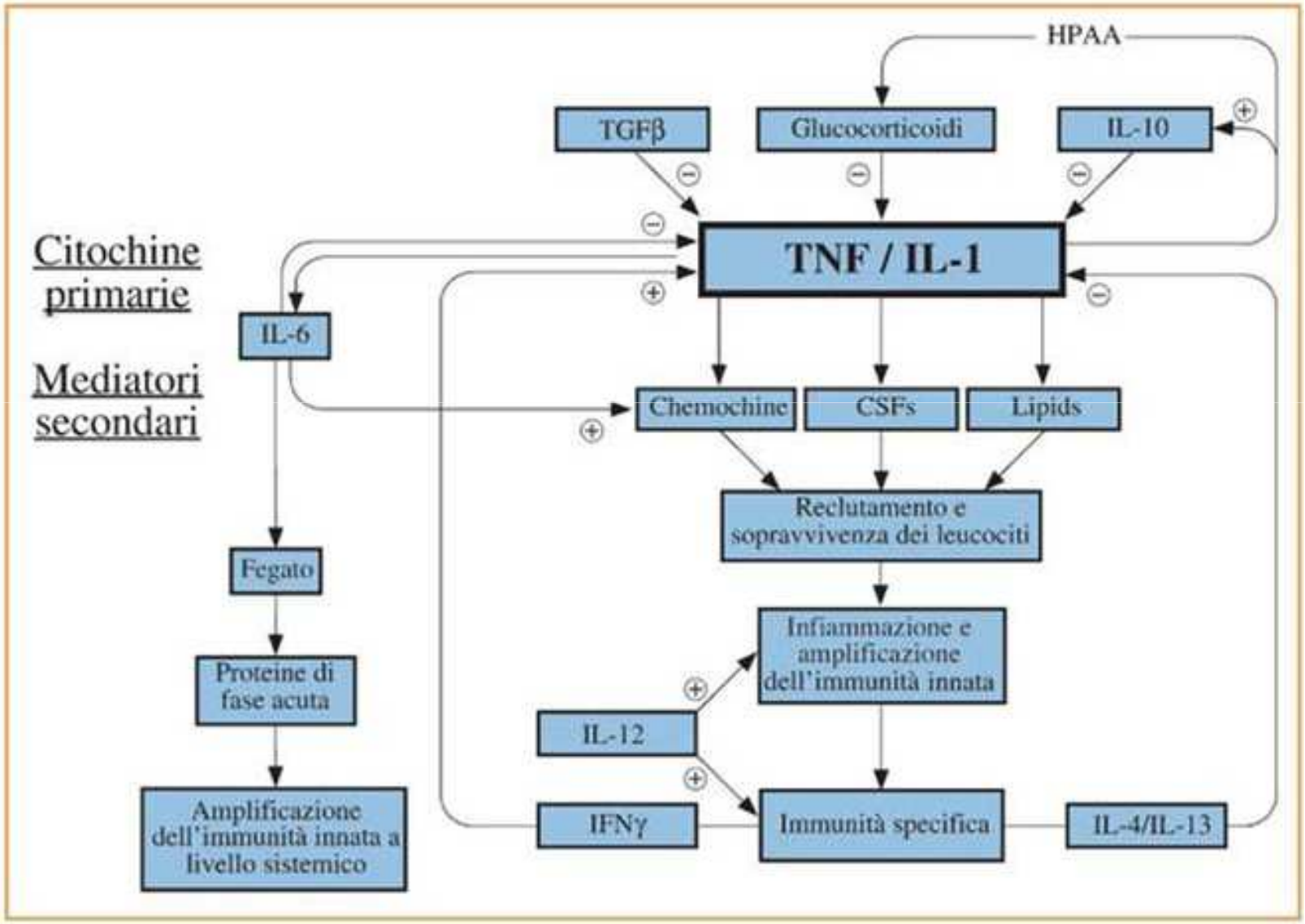
Received 9 August 1993; Available online 30 November 2004.

Summary

Interleukin-10 (IL-10) affects the growth and differentiation of many hemopoietic cells in vitro; in particular, it is a potent suppressor of macrophage and T cell functions. In IL-10-deficient mice, generated by gene targeting, lymphocyte development and antibody responses are normal, but most animals are growth retarded and anemic and suffer from chronic enterocolitis. Alterations in intestine include extensive mucosal hyperplasia, inflammatory reactions, and aberrant expression of major histocompatibility complex class II molecules on epithelia. In contrast, mutants kept under specific pathogen-free conditions develop only a local inflammation limited to the proximal colon. These results indicate that the bowel inflammation in the mutants originates from uncontrolled immune responses stimulated by enteric antigens and that IL-10 is an essential immunoregulator in the intestinal tract.


HPAA
TGFβ
Glucocorticoidi
IL-10
Citochine primarie
TNF / IL-1
IL-6
Mediatori secondari
Chemochine
CSFs
Lipids
Reclutamento e sopravvivenza dei leucociti
Fegato
Infiammazione e amplificazione dell'immunità innata
Proteine di fase acuta
IL-12
Amplificazione dell'immunità innata a livello sistemico
IFNγ
Immunità specifica
IL-4/IL-13

Gut 2001;49:42–46

Interleukin 10 (Tenovil) in the prevention of postoperative recurrence of Crohn's disease

J-F Colombel, P Rutgeerts, H Malchow, M Jacyna, O H Nielsen, J Rask-Madsen, S Van Deventer, A Ferguson, P Desreumaux, A Forbes, K Geboes, L Melani, M Cohard

Abstract

***Background and aims*—New lesions of Crohn's disease occur early after ileal or ileocolonic resection and ileocolonic anastomosis. We performed a double blind controlled trial to evaluate the safety and tolerance of recombinant human interleukin 10 (IL-10; Tenovil) in subjects operated on for Crohn's disease. We also assessed the effect of Tenovil in preventing endoscopic recurrence 12 weeks after surgery.**

***Methods*—Patients with Crohn's disease who underwent curative ileal or ileocolonic resection and primary anastomosis were randomised within two weeks after surgery to receive subcutaneous Tenovil 4 µg/kg once daily (QD) (n=22) or 8 µg/kg twice weekly (TIW) (n=21), or placebo (QD or TIW) (n=22). An ileocolonoscopy was performed after 12 weeks of treatment.**

***Results*—Compliance was excellent. The most frequently observed adverse events were mild and moderate in severity and equally distributed across treatment groups. Thirty seven patients in the pooled Tenovil group and 21 patients in the pooled placebo group were evaluable by endoscopy. At 12 weeks, 11 of 21 patients (52%) in the placebo group had recurrent lesions compared with 17 of 37 patients (46%) in the Tenovil group (ns). The incidence of severe endoscopic recurrence was similar in both groups (9%).**

***Conclusion*—Tenovil treatment for 12 consecutive weeks in patients with Crohn's disease after intestinal resection was safe and well tolerated. No evidence of prevention of endoscopic recurrence of Crohn's disease by Tenovil was observed.**

(*Gut* 2001;49:42–46)

Table 2 Endoscopic score at 12 weeks: intent to treat population (n=65)

Score	*Tenovil 4 µg/kg QD (n=22)*	*Tenovil 8 µg/kg TIW (n=21)*	*Pooled Tenovil (n=43)*	*Pooled placebo (n=22)*
i0	11 (50%)	9 (43%)	20 (46%)	10 (45%)
i1+i2	3 (14%)	11 (52%)	14 (30%)	9 (41%)
i3+i4	2 (9%)	1 (5%)	3 (9%)	2 (9%)
Missing	6 (27%)*	—	6 (14%)*	1 (5%)†

*These subjects did not have a rating at treatment end point for the following reasons: two subjects did not meet protocol eligibility; two subjects experienced an adverse event; one subject had an anatomical problem with regard to endoscopy; and one subject did not wish to continue the study.

†One subject experienced an adverse event during randomisation and did not receive the study medication.

molecular pharmaceutics

ARTICLE

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Treatment of Mice with Dextran Sulfate Sodium-Induced Colitis with Human Interleukin 10 Secreted by Transformed *Bifidobacterium longum*

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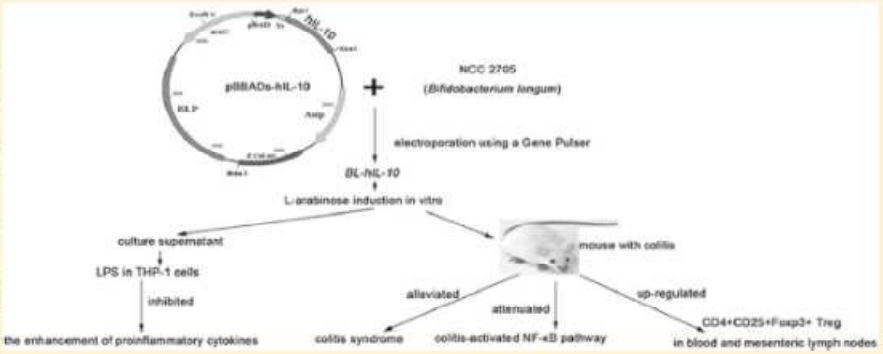
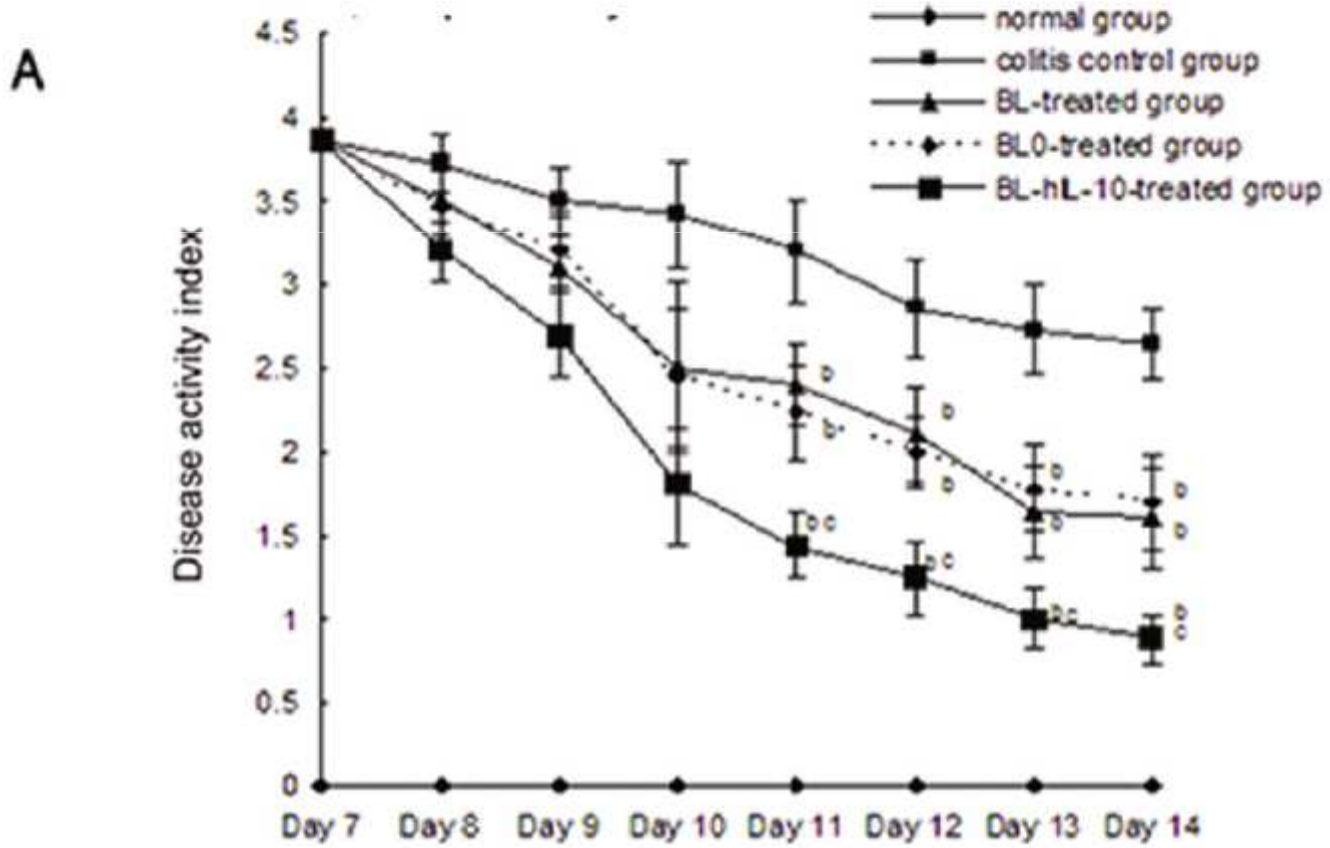
ABSTRACT: Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) the etiology of which has not yet been fully clarified. Cytokine interleukin-10 (IL-10) plays a central role in downregulating inflammatory cascade in UC and is likely a candidate for therapeutic intervention. However, its intravenous administration is costly and inconvenient. Therefore, we established a novel IL-10 delivery system by transforming a hIL-10-containing plasmid into *B. longum* (*BL-hIL-10*) and investigated its effects on 5% dextran sulfate sodium (DSS)-induced ulcerative colitis in mice and the possible underlying mechanism. Our results show that (1) hIL-10 was expressed and secreted into the culture supernatant of *BL-hIL-10* after L-arabinose induction *in vitro* as examined by Western blot, enzyme-linked immunosorbent assay (ELISA) and RT-PCR; (2) addition of *BL-hIL-10* culture supernatant had no cytotoxic effect and morphological alteration, but significantly inhibited the enhancement of proinflammatory cytokines by lipopolysaccharide (LPS) in THP-1 cells; (3) oral administration of *BL-hIL-10* alleviated colitis syndrome of the model mice, attenuated colitis-activated NF-κB pathway measured by DNA-binding assay and colitis-elevated expression of proinflammatory cytokines examined with CCK cytotoxic kits, and upregulated $CD4^+CD25^+Foxp3^+$ Treg in blood and mesenteric lymph nodes measured by flow cytometry. In conclusion, *BL-hIL-10* as a novel oral hIL-10 delivery system has been successfully established and oral administration of *BL-hIL-10* alleviated inflammatory damage of colonic tissue in the model mice by blocking the colitis-activated NF-κB pathway and upregulating $CD4^+CD25^+Foxp3^+$ Treg in blood and mesenteric lymph nodes in mice.

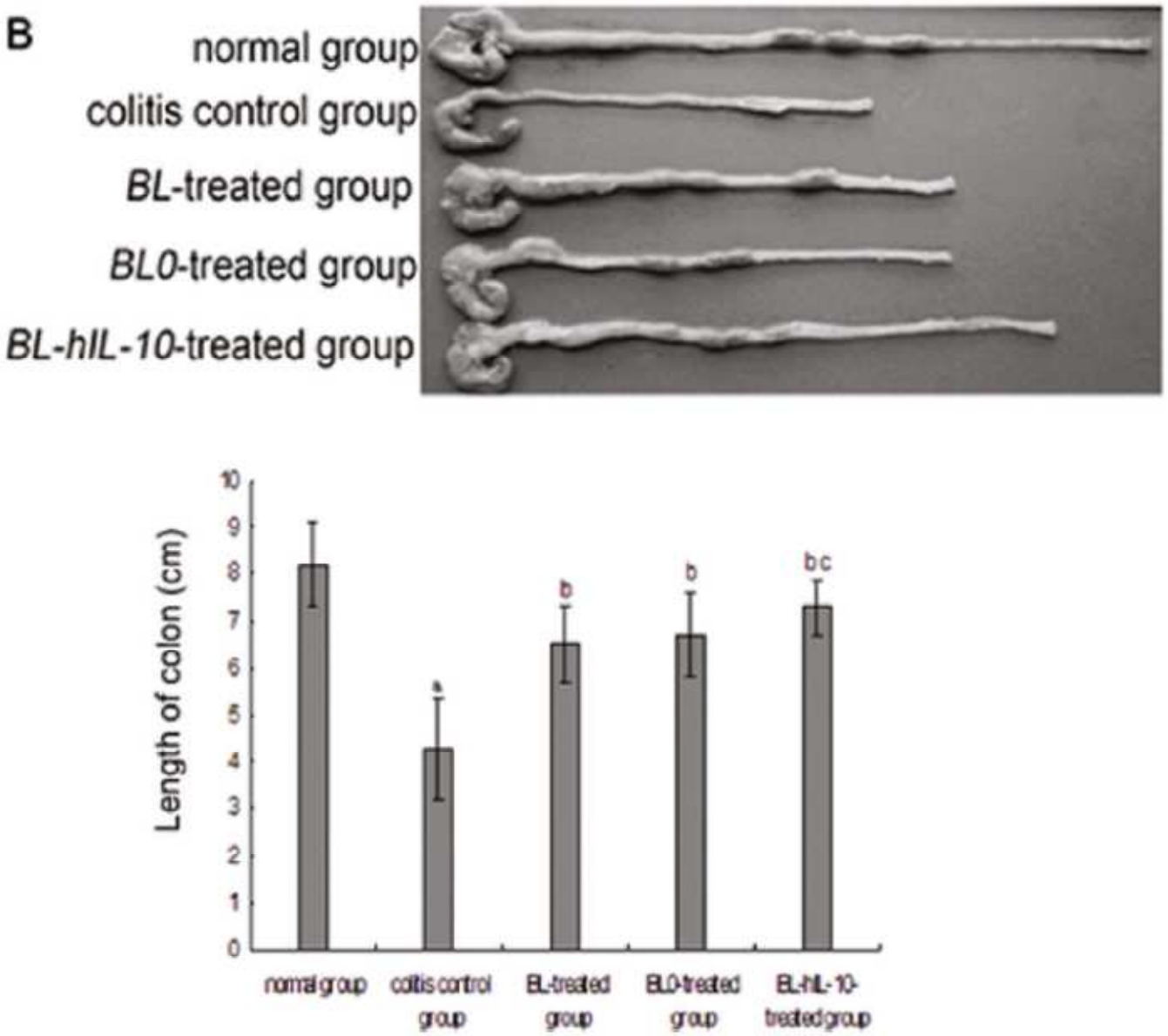


KEYWORDS: ulcerative colitis, *Bifidobacterium longum*, *BL-hIL-10*, interleukin 10

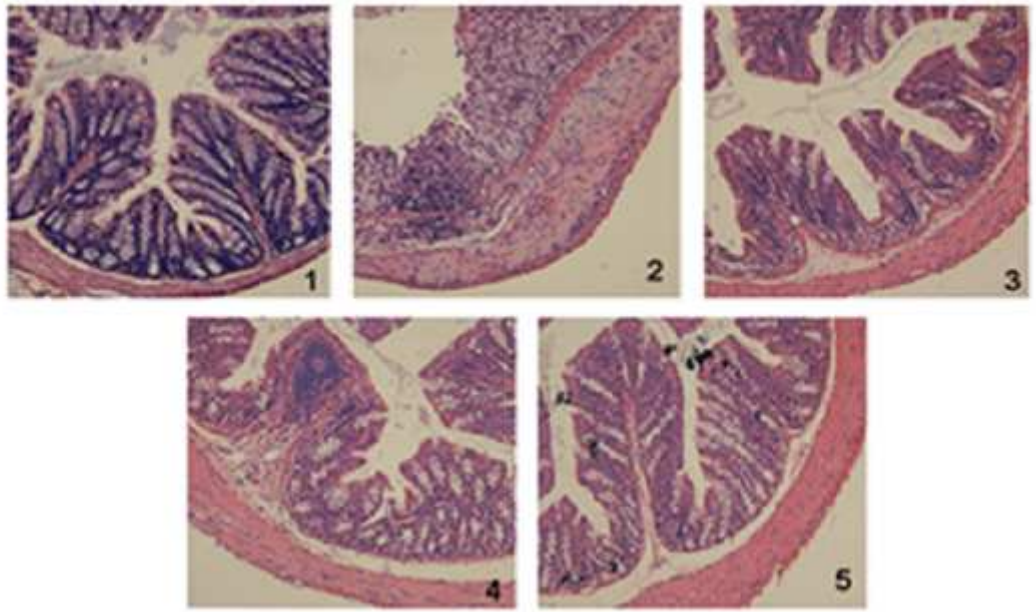
Table 2. Disease Activity Index Scoring System

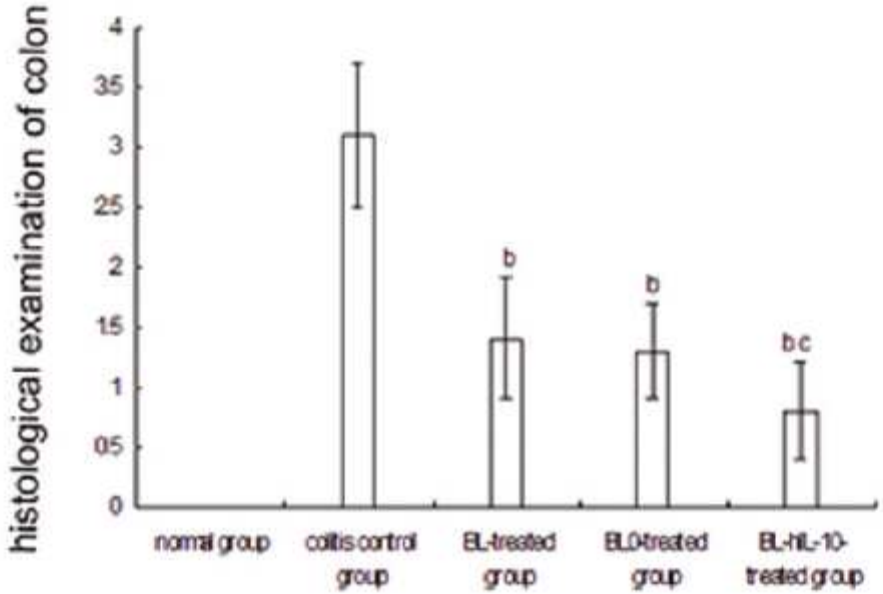
score	weight loss	stool consistency	occult/bloody stools
0	−	normal	normal
1	1−5%		
2	6−10%	loose	occult +
3	11−15%		
4	>15%	diarrhea	bloody stools

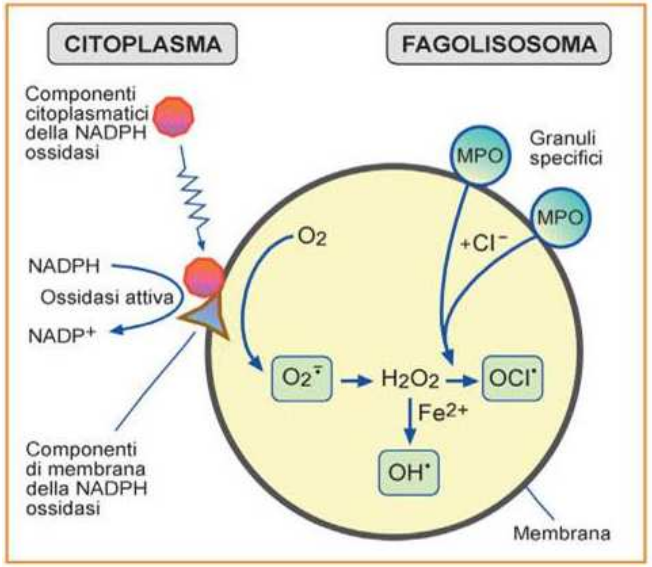



B
normal group
colitis control group
BL-treated group
BL0-treated group
BL-hIL-10-treated group
Length of colon (cm)
0
1
2
3
4
5
6
7
8
9
10
a
b
b
bc
normal group
colitis control group
BL-treated group
BL0-treated group
BL-hIL-10-treated group

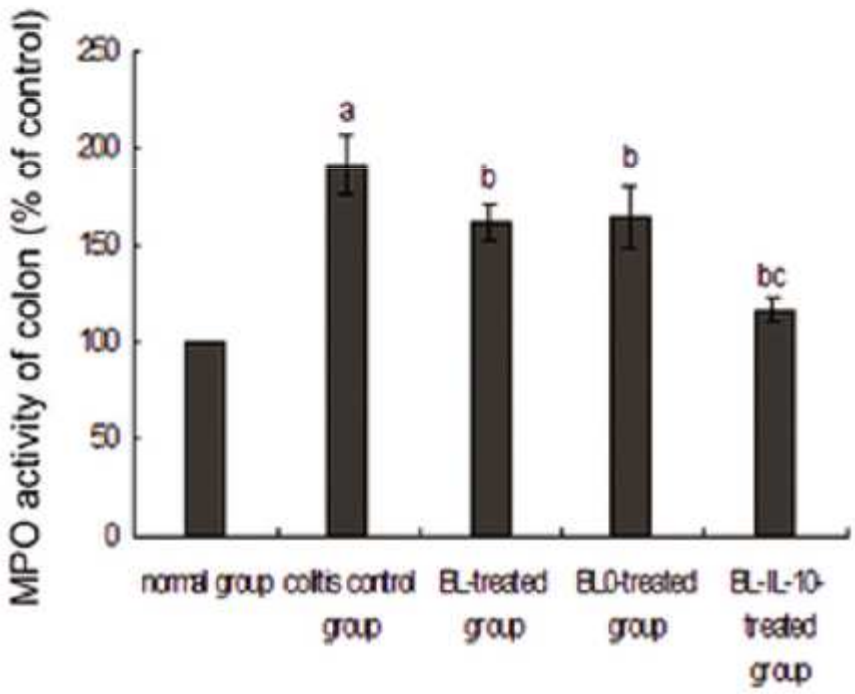
C


1
2
3
4
5


histological examination of colon
4
35
3
25
2
15
1
05
0
b
b
bc
normal group
colitis control group
BL-treated group
BL0-treated group
BL-hIL-10-treated group


CITOPLASMA
FAGOLISOSOMA
Componenti citoplasmatici della NADPH ossidasi
MPO
Granuli specifici
MPO
O2
+Cl⁻
NADPH
Ossidasi attiva
NADP+
O2⁻
H2O2
OCl⁻
Fe2+
OH⁻
Componenti di membrana della NADPH ossidasi
Membrana

D


MPO activity of colon (% of control)
250
200
150
100
50
0
a
b
b
bc
normal group
colitis control group
BL-treated group
BL0-treated group
BL-IL-10-treated group

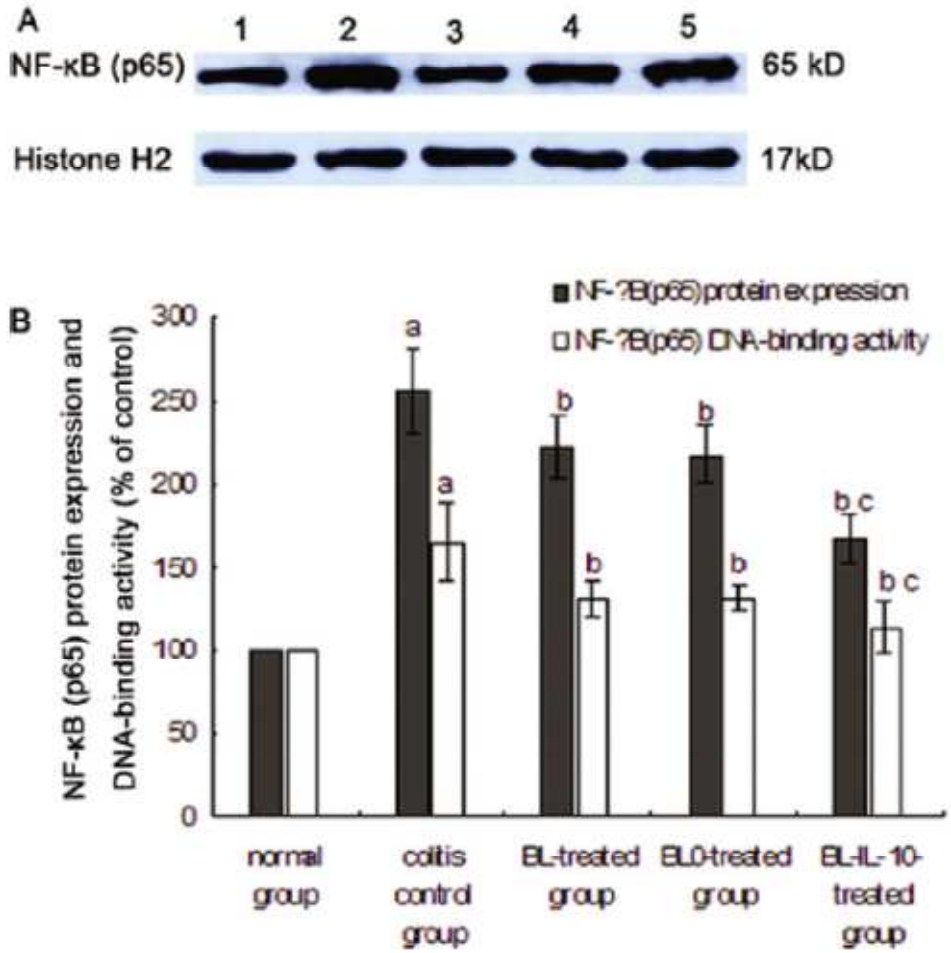
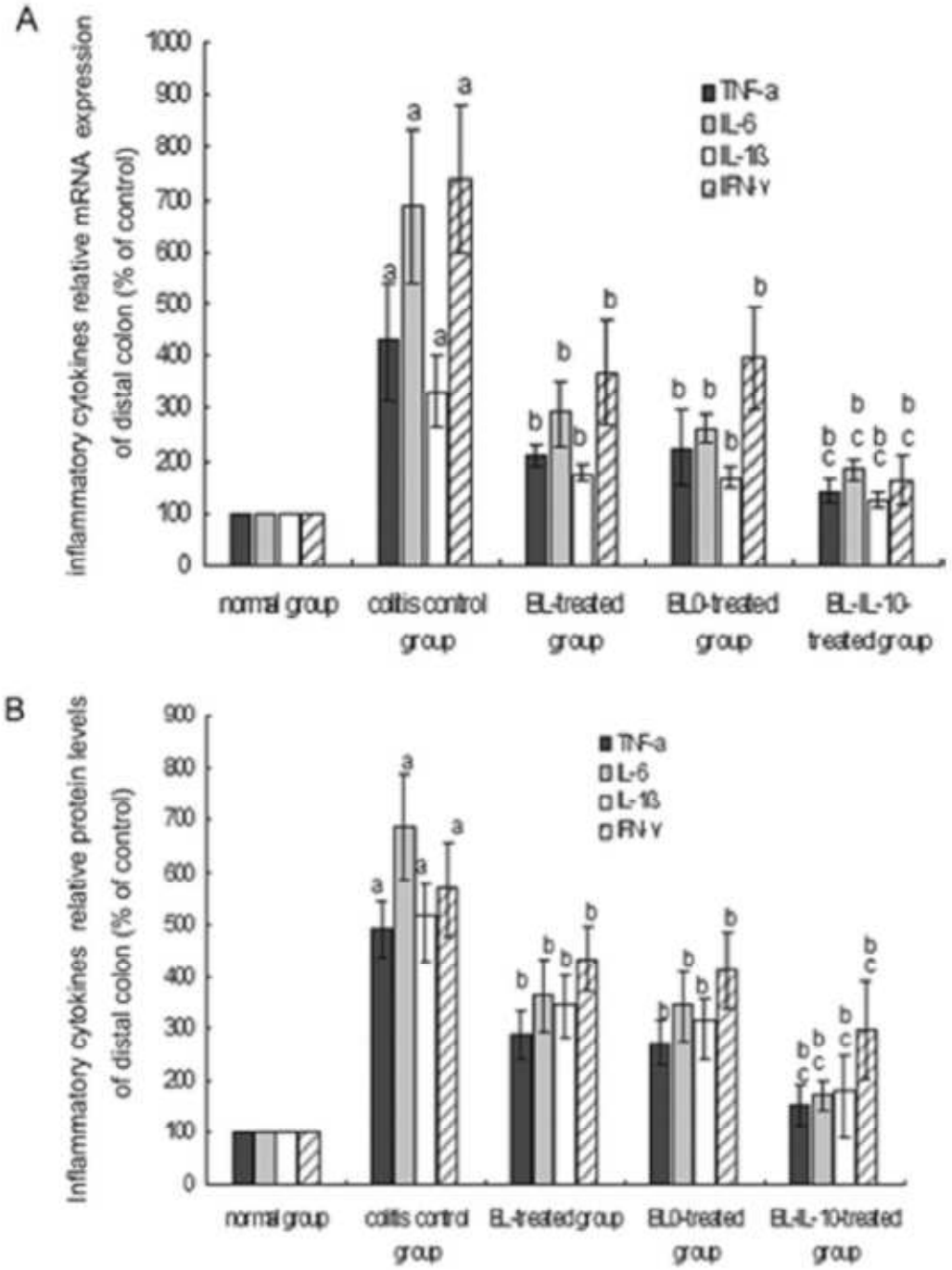
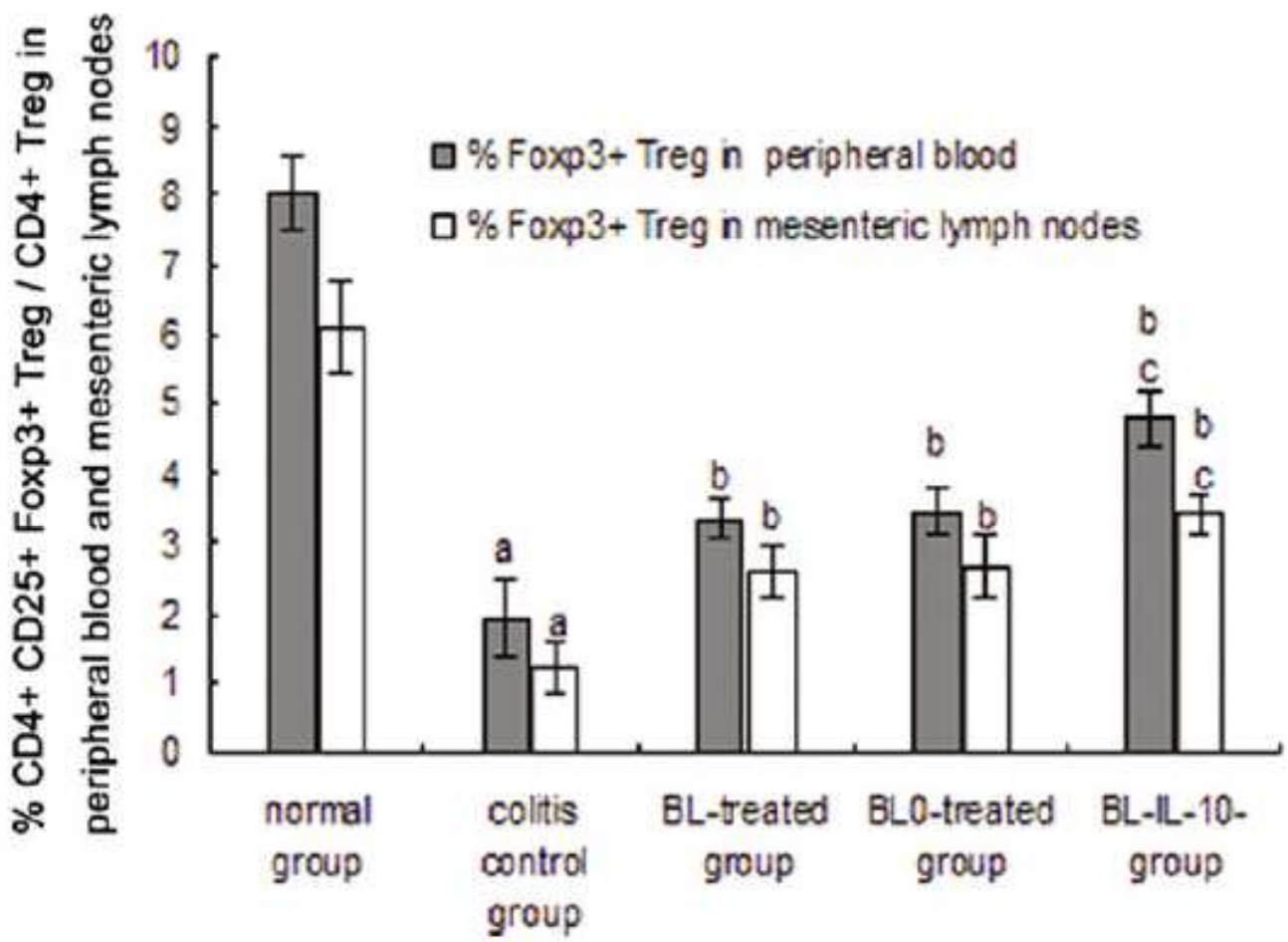


Figure 5. The effects of *BL-IL-10* on NF-κB (p65) protein expression and DNA-binding activity of distal colon in mice. A: NF-κB (p65) protein expression in (1) normal mice; (2) mice with colitis; (3) mice with colitis treated with *BL-IL-10*; (4) mice with colitis treated with *BL*; (5) mice with colitis treated with *BL0*. B: Relative NF-κB (p65) protein expression and DNA-binding activity. Letters a, b and c indicate $p < 0.05$ compared with those in normal mice, mice with colitis and mice with colitis treated with *BL*, respectively.


A
Inflammatory cytokines relative mRNA expression of distal colon (% of control)
1000
900
800
700
600
500
400
300
200
100
0
TNF-a
IL-6
IL-18
IFN-γ
normal group
colitis control group
EL-treated group
ELD-treated group
EL-IL-10-treated group
B
Inflammatory cytokines relative protein levels of distal colon (% of control)
900
800
700
600
500
400
300
200
100
0
TNF-a
IL-6
IL-18
IFN-γ
normal group
colitis control group
EL-treated group
ELD-treated group
EL-IL-10-treated group


% CD4+ CD25+ Foxp3+ Treg / CD4+ Treg in peripheral blood and mesenteric lymph nodes
10
9
8
7
6
5
4
3
2
1
0
% Foxp3+ Treg in peripheral blood
% Foxp3+ Treg in mesenteric lymph nodes
a
a
b
b
b
b
b
c
b
c
normal group
colitis control group
BL-treated group
BL0-treated group
BL-IL-10-group