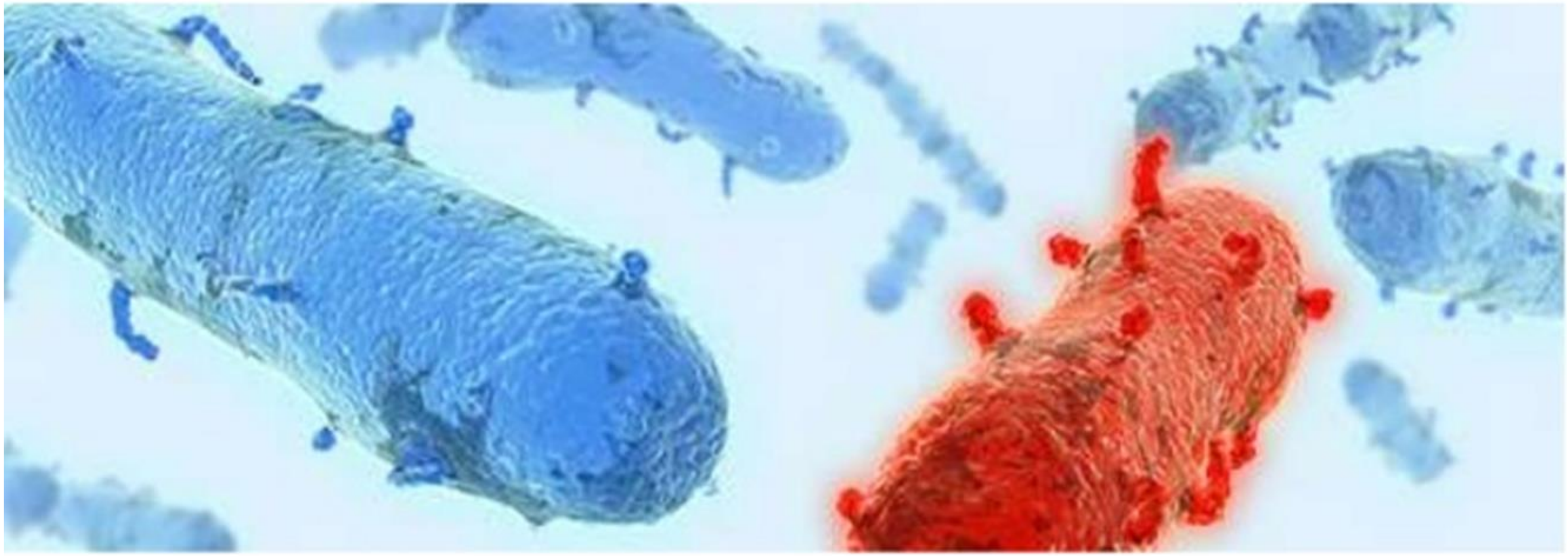
Technological
Educational
Institute of Athens

Molecular biology and Microbiology



Department of Medical laboratories

Theodoros Rampias
19/06/ 2017

Next Generation Sequencing

Applied and Environmental Microbiology, June 2011, p. 3846–3852 Vol. 77, No. 11
0099-2240/11/$12.00 doi:10.1128/AEM.02772-10

Generation of Multimillion-Sequence 16S rRNA Gene Libraries from Complex Microbial Communities by Assembling Paired-End Illumina Reads▽†

Andrea K. Bartram,[1] Michael D. J. Lynch,[2] Jennifer C. Stearns,[1] Gabriel Moreno-Hagelsieb,[2] and Josh D. Neufeld[1]*

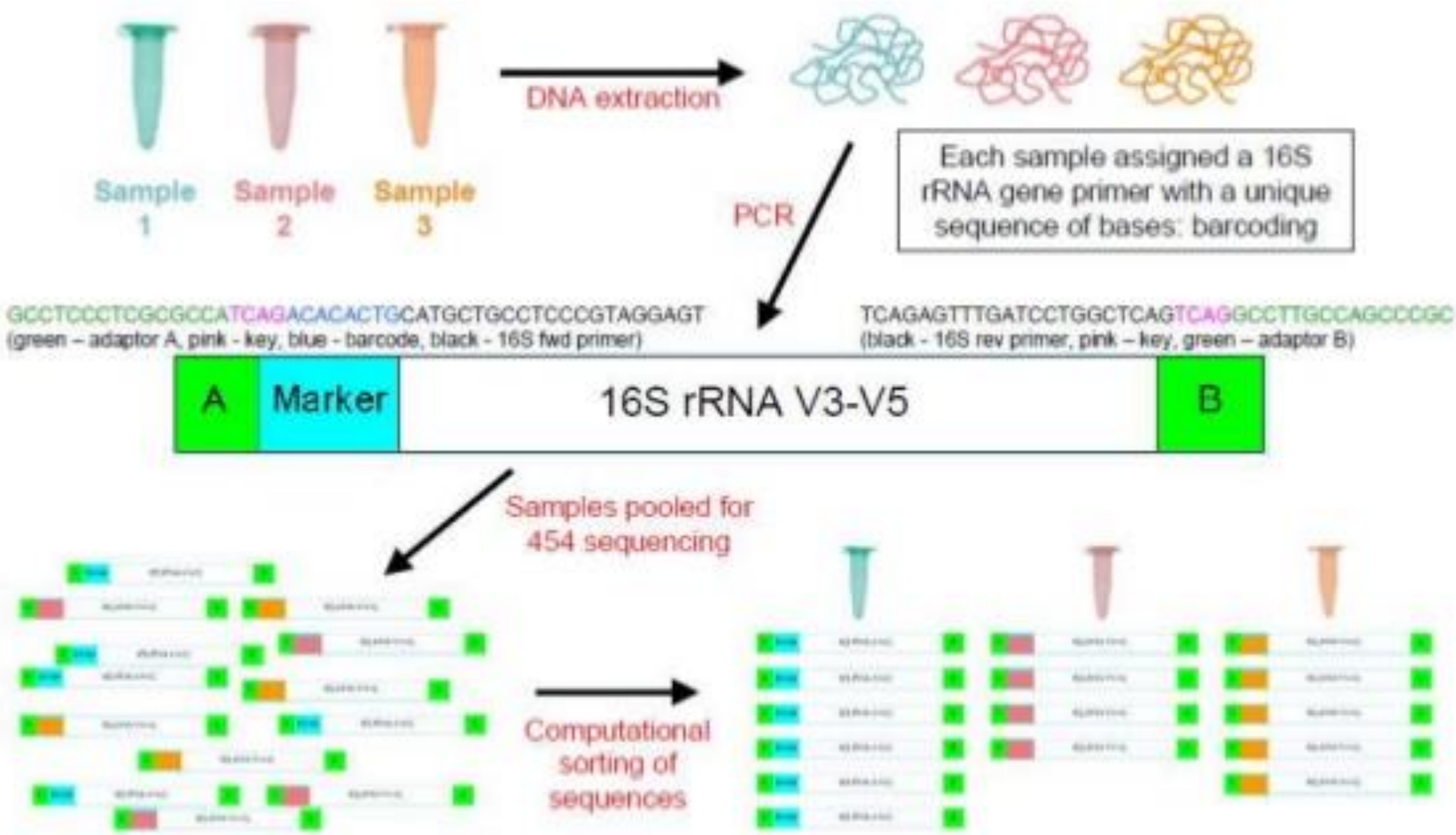
Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1,[1] and Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada N2L 3C5[2]

Received 25 November 2010/Accepted 24 March 2011

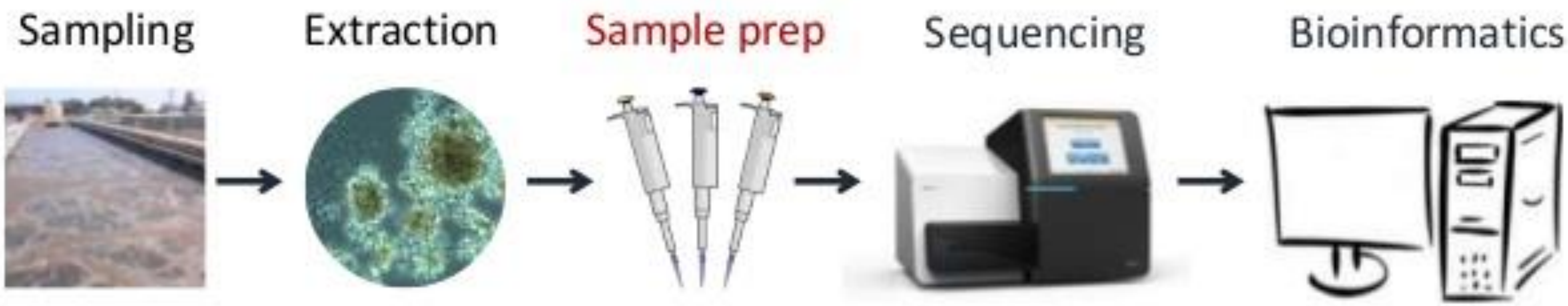
Microbial communities host unparalleled taxonomic diversity. Adequate characterization of environmental and host-associated samples remains a challenge for microbiologists, despite the advent of 16S rRNA gene sequencing. In order to increase the depth of sampling for diverse bacterial communities, we developed a method for sequencing and assembling millions of paired-end reads from the 16S rRNA gene (spanning the V3 region; ~200 nucleotides) by using an Illumina genome analyzer. To confirm reproducibility and to identify a suitable computational pipeline for data analysis, sequence libraries were prepared in duplicate for both a defined mixture of DNAs from known cultured bacterial isolates (>1 million postassembly sequences) and an Arctic tundra soil sample (>6 million postassembly sequences). The Illumina 16S rRNA gene libraries represent a substantial increase in number of sequences over all extant next-generation sequencing approaches (e.g., 454 pyrosequencing), while the assembly of paired-end 125-base reads offers a methodological advantage by incorporating an initial quality control step for each 16S rRNA gene sequence. This method incorporates indexed primers to enable the characterization of multiple microbial communities in a single flow cell lane, may be modified readily to target other variable regions or genes, and demonstrates unprecedented and economical access to DNAs from organisms that exist at low relative abundances.

Now generate V3-V4 bacterial amplicons (~450 bases)
Usually PE 300

454-based 16S amplicon sequencing

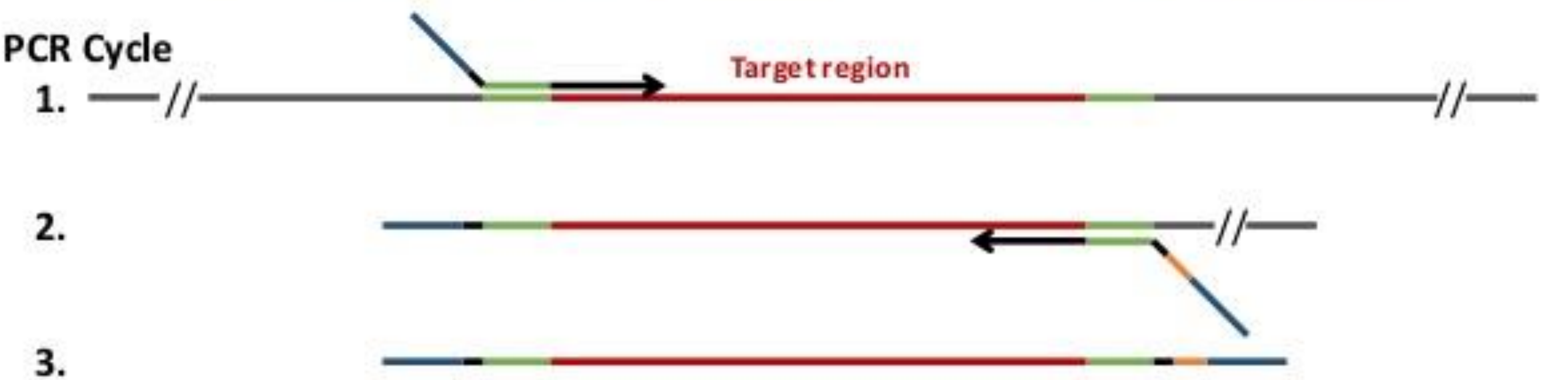


Typical workflow

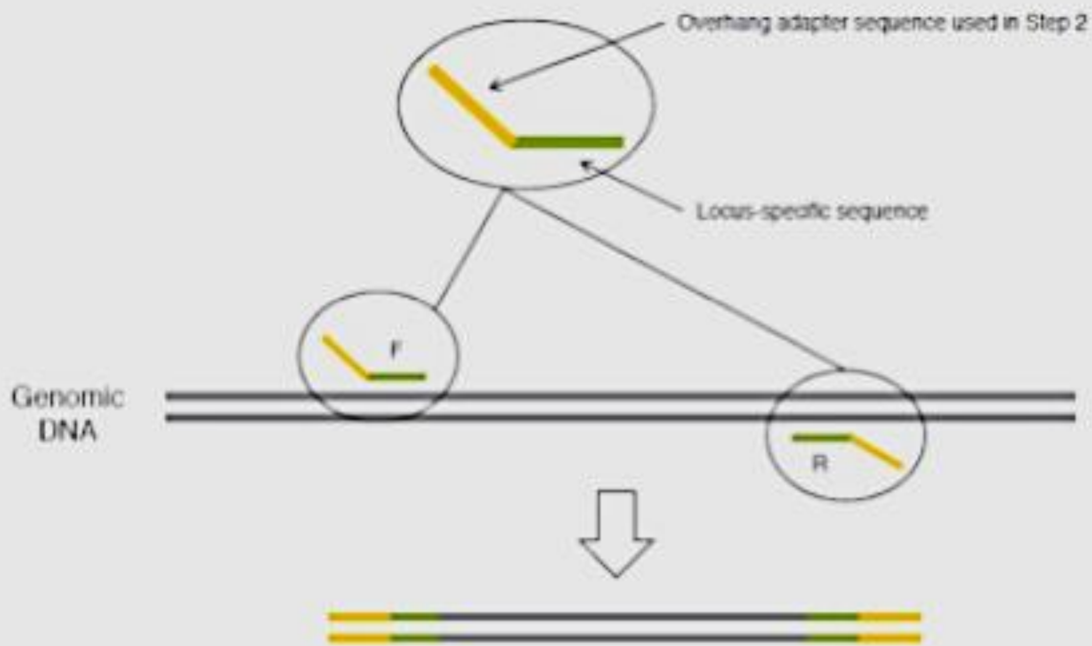


PCR with modified 16S primers

Illumina adapter	Barcode	Pad	linker	Primer
5′-AATGATACGGCGACCACCGAGATCTACAC		GTACGTACG	GT	27F: AGAGTTTGATCCTGGCTCAG-3′
5′-CAAGCAGAAGACGGCATACGAGAT	TCCCTTGTCTCC	ACGTACGTAC	CCG	534R: ATTACCGCGGCTGCTGG-3′



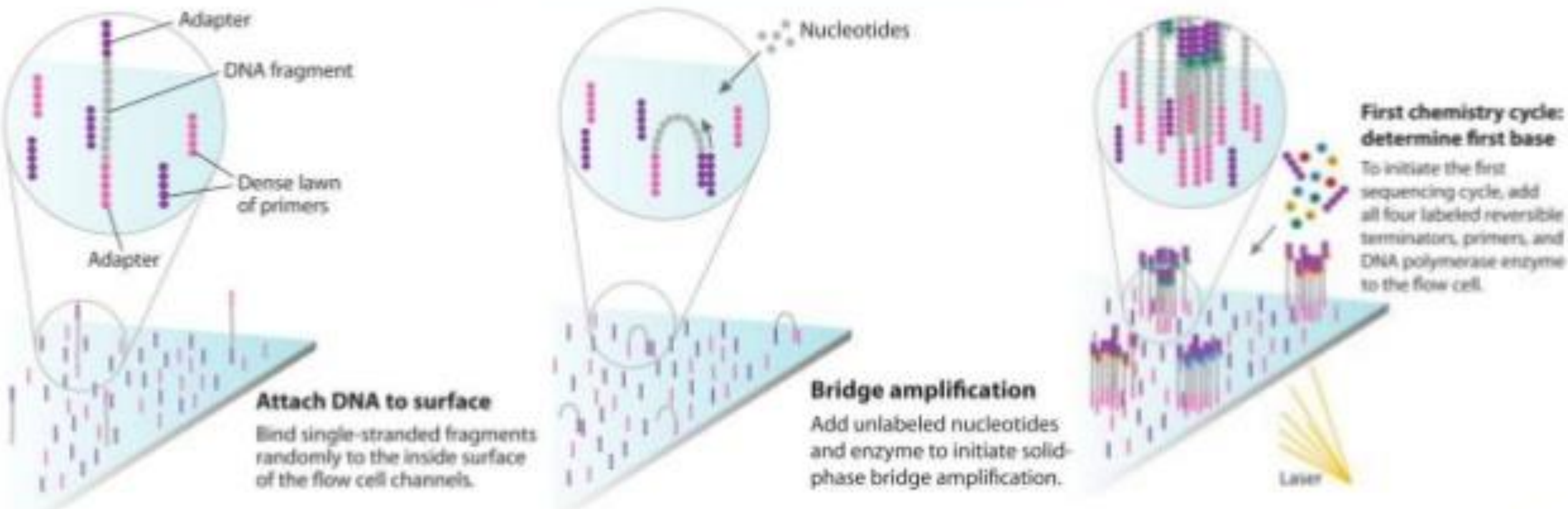
16S Amplicon



Typical workflow

Sampling → Extraction → Sample prep → Sequencing → Bioinformatics

≈ 500 bp target amplicon

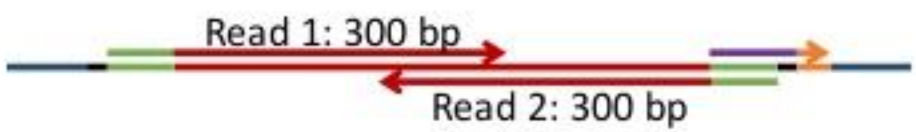


Mardis, 2008 (PMID 18576944)

Typical workflow

Sampling | Extraction | Sample prep | Sequencing | Bioinformatics

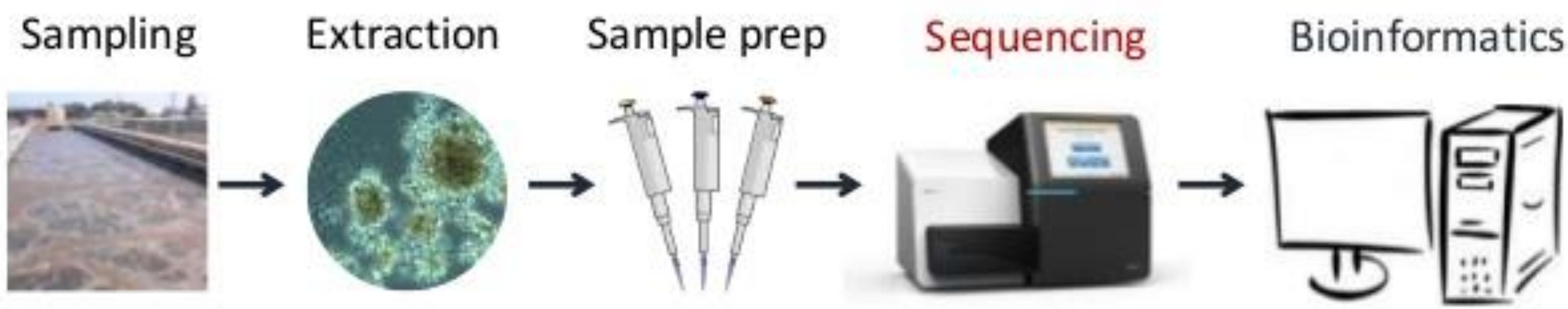
≈ 500 bp target amplicon



After Sequencing:

Read 1
Read 2
Barcode

Typical workflow

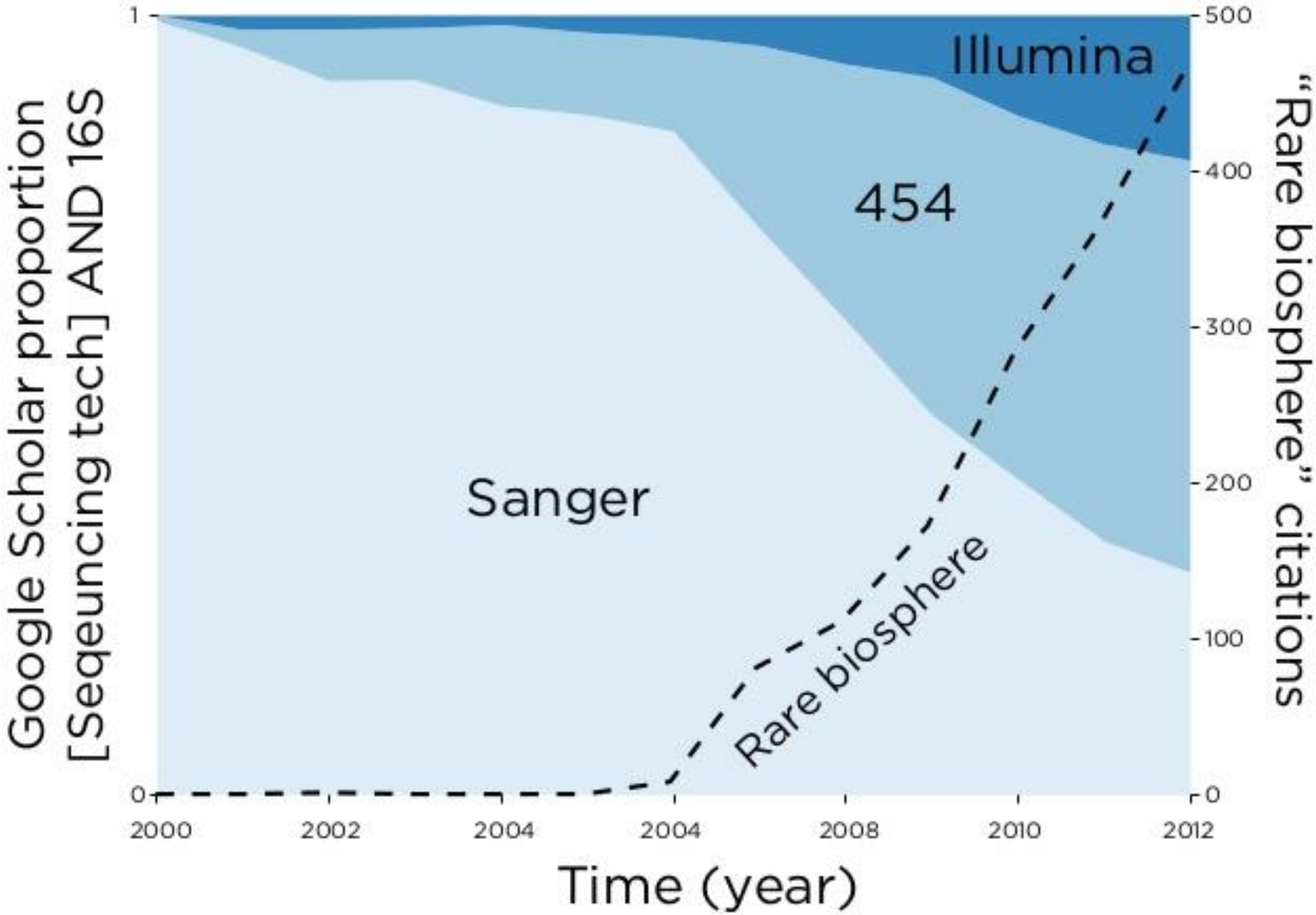


How many sequences are needed? It depends on your question!

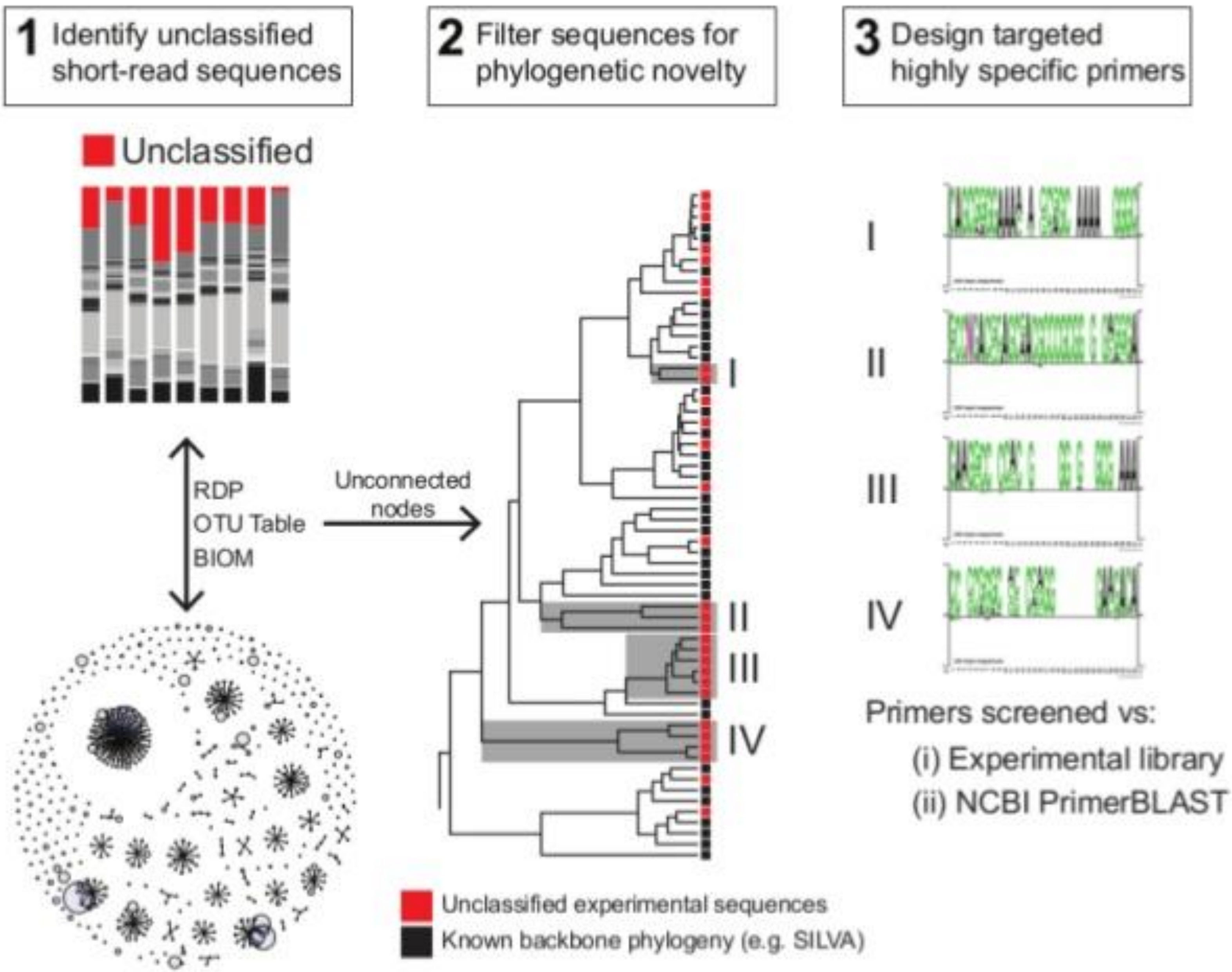
(although 50.000 raw sequences per sample is usually fine)

AAU raw kit and chemical costs (DKK)	Cost	Cost v2
DNA extraction	105	70[a]
Library preparation	40	40
Sequencing (min 100k reads / sample)	190[b]	70[c]
Total	**335**	**180**

[a] Kits discounted
[b] 50 samples per run
[c] 150 samples per run (can run up to 300)



Lynch and Neufeld. 2013. Nat. Rev. Microbiol. In preparation.


1 Identify unclassified short-read sequences
2 Filter sequences for phylogenetic novelty
3 Design targeted highly specific primers
Unclassified
RDP
OTU Table
BIOM
Unconnected nodes
I
II
III
IV
I
II
III
IV
Primers screened vs:
(i) Experimental library
(ii) NCBI PrimerBLAST
Unclassified experimental sequences
Known backbone phylogeny (e.g. SILVA)

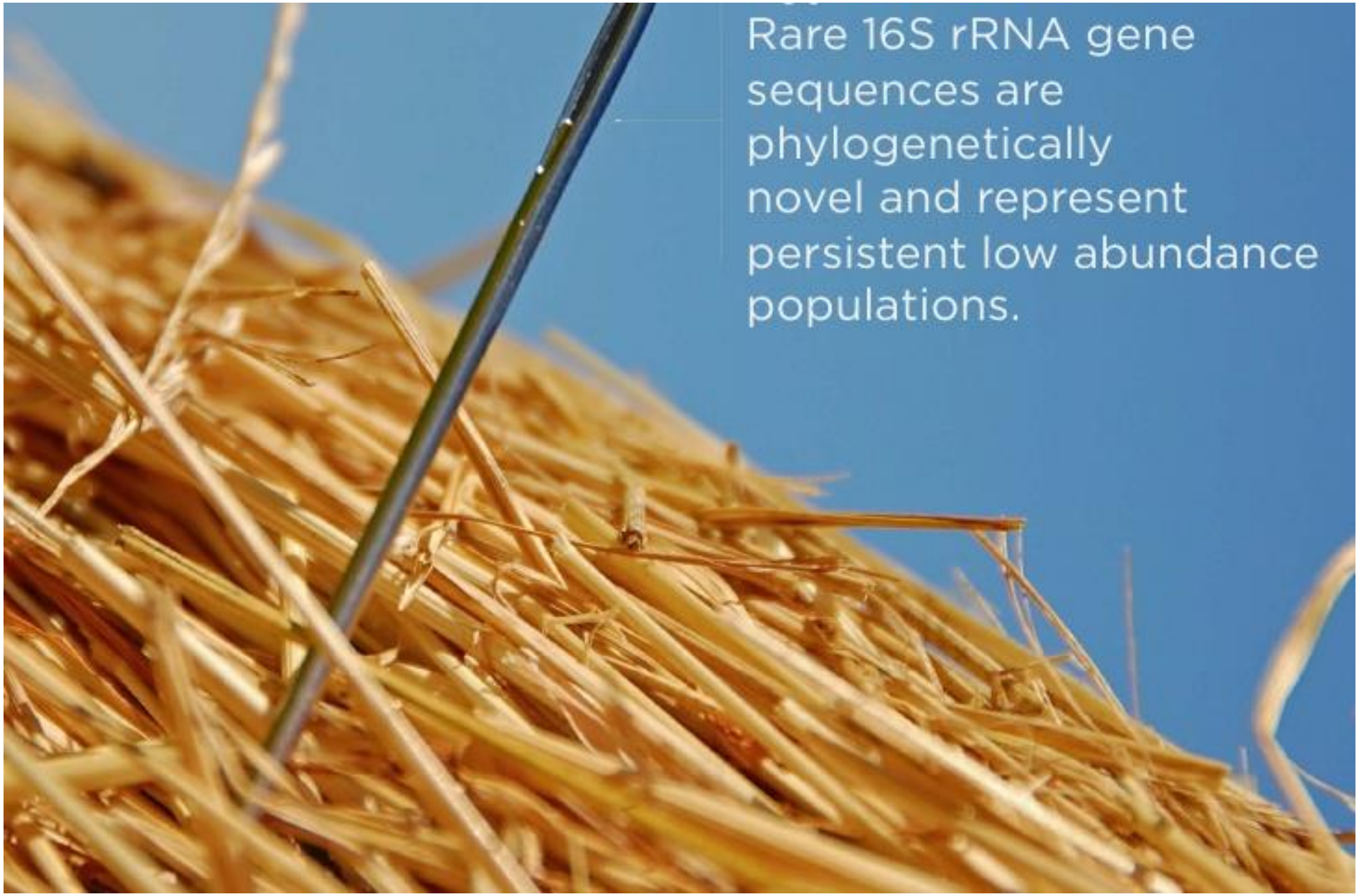
SAMPLING MATTERS……….

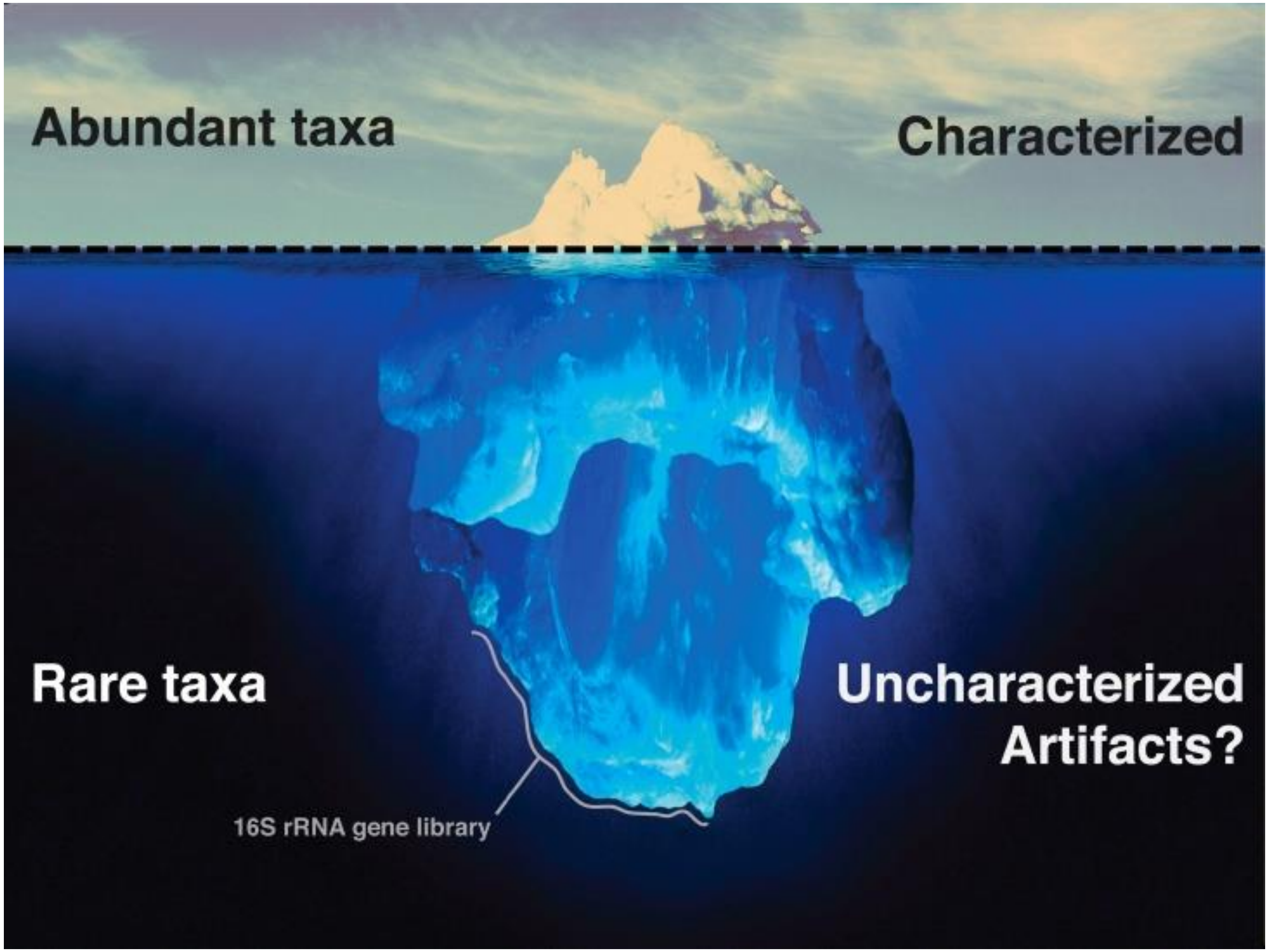
Microbial community resemblance methods differ in their ability to detect biologically relevant patterns

NATURE METHODS | VOL.7 NO.10 | OCTOBER 2010 | 813

Justin Kuczynski[1], Zongzhi Liu[2], Catherine Lozupone[3], Daniel McDonald[3], Noah Fierer[4,5] & Rob Knight[3,6]

"The advantages of having large numbers of samples at shallow coverage (~1,000 sequences per sample) clearly outweigh having a small number of samples at greater coverage for many datasets, suggesting that the focus for future studies should be on broader sampling that can reveal association with key biological parameters rather than on deeper sequencing."


Rare 16S rRNA gene
sequences are
phylogenetically
novel and represent
persistent low abundance
populations.


Abundant taxa
Characterized
Rare taxa
Uncharacterized
Artifacts?
16S rRNA gene library

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

High-throughput sequencing and clinical microbiology: progress, opportunities and challenges

Mark J Pallen, Nicholas J Loman and Charles W Penn

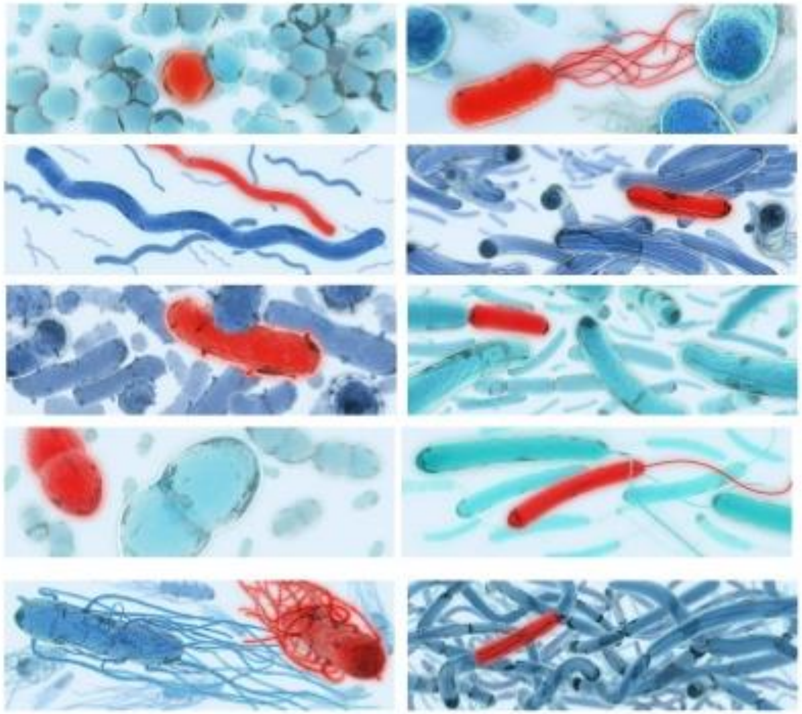
High-throughput sequencing is sweeping through clinical microbiology, transforming our discipline in its wake. It is already providing an enhanced view of pathogen biology through rapid and inexpensive whole-genome sequencing and more sophisticated applications such as RNA-seq. It also promises to deliver high-resolution genomic epidemiology as the ultimate typing method for bacteria. However, the most revolutionary effect of this 'disruptive technology' is likely to be creation of a novel sequence-based, culture-independent diagnostic microbiology that incorporates microbial community profiling, metagenomics and single-cell genomics. We should prepare for the coming 'technological singularity' in sequencing, when this technology becomes so fast and so cheap that it threatens to out-compete existing diagnostic and typing methods in microbiology.

Address
School of Biosciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom

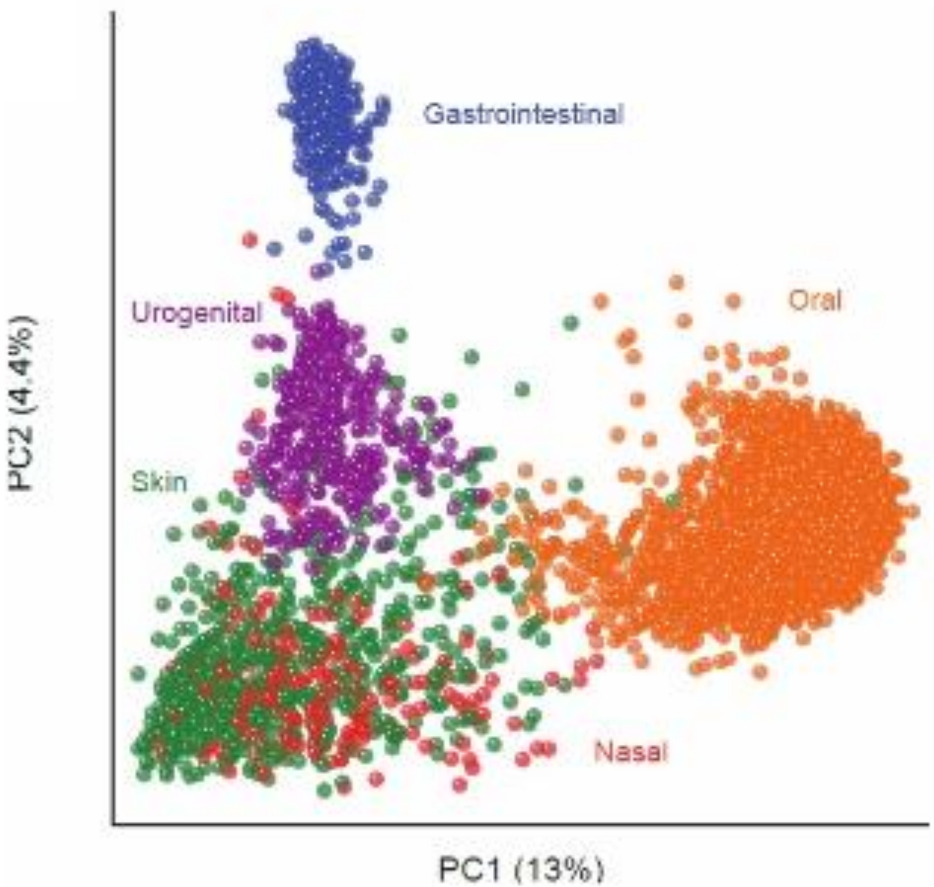
http://pathogenomics.bham.ac.uk/blog/2011/08/are-diagnostic-and-public-health-bacteriology-ready-to-become-branches-of-genomic-medicine/

- NGS

Gut Microbiome



Microorganisms cluster by body site



<u>Cataloguing efforts by the NIH Human microbiome project suggest:</u>

~10,000 organisms live with us

~ 8×10^6 genes in this "second genome"

<u>Identifying microbiota in healthy individuals revealed:</u>

Different body sites have unique communities

Race, Age, Gender, Weight or Ethnicity have no effect

NIH Human Microbiome Project

Profiling 5 body sites

- Nasal
- Mouth
- Skin
- Gastrointestinal system
- Urogential

Compare between individuals:

- Healthy vs. Disease
- Treated vs. Untreated
- Twin studies
- Diet
- …

- **Gut**
  - Intestinal infections
  - Obesity
  - Inflammatory Bowel Disease
- **Airway**
  - Pneumonia and other respiratory infections
  - Chronic Obstructive Pulmonary Disease
  - Cystic Fibrosis
- **Urogenital**
  - Bacterial Vaginosis
  - Urinary Tract Infections
  - Sexually Transmitted Disease
- **Blood**
  - Sepsis/Blood-stream infections
- **Cancer**
- **Heart disease**
- **Neurological disorders**
- **Oral**
  - Periodontitis
  - Gingivitis

WHAT DO THE MICROBES DO FOR US?

- Provide the ability to harvest nutrients and
- Produce additional energy otherwise inaccessible to the host.
- Produce vitamins
- Metabolize xenobiotics
- Provide resistance to tumor and cancer leading neoplasms
- Assist in developing a mature immune system

Benefits of the normal flora

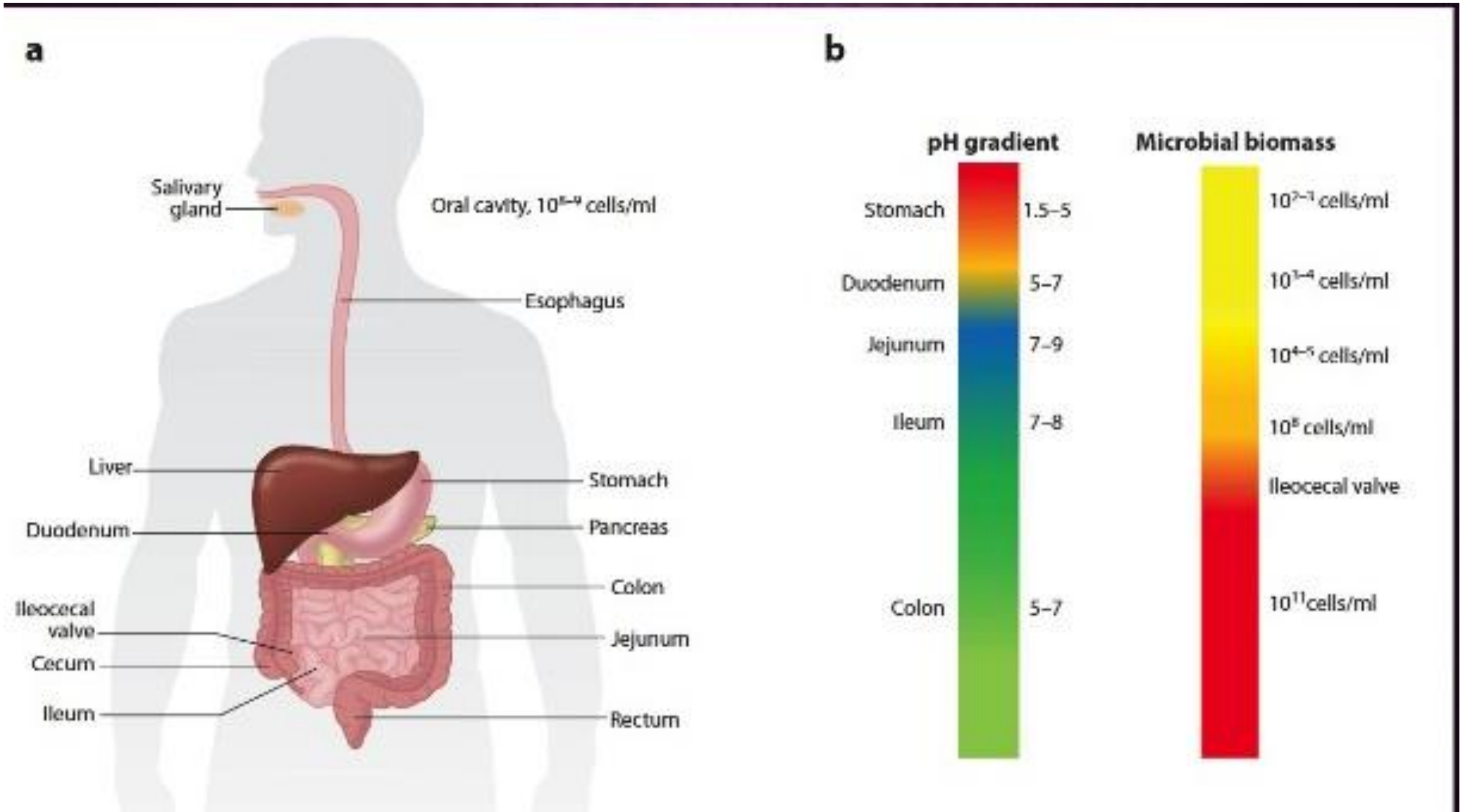
1. **Synthesize and excrete vitamins**
   Vitamin K and Vitamin B12

2. **Prevent colonization by pathogens**
   competing for attachment sites or for essential nutrients

3. **May antagonize other bacteria**
   the production of substances which inhibit or kill non-indigenous species(nonspecific fatty acids, peroxides, bacteriocins).

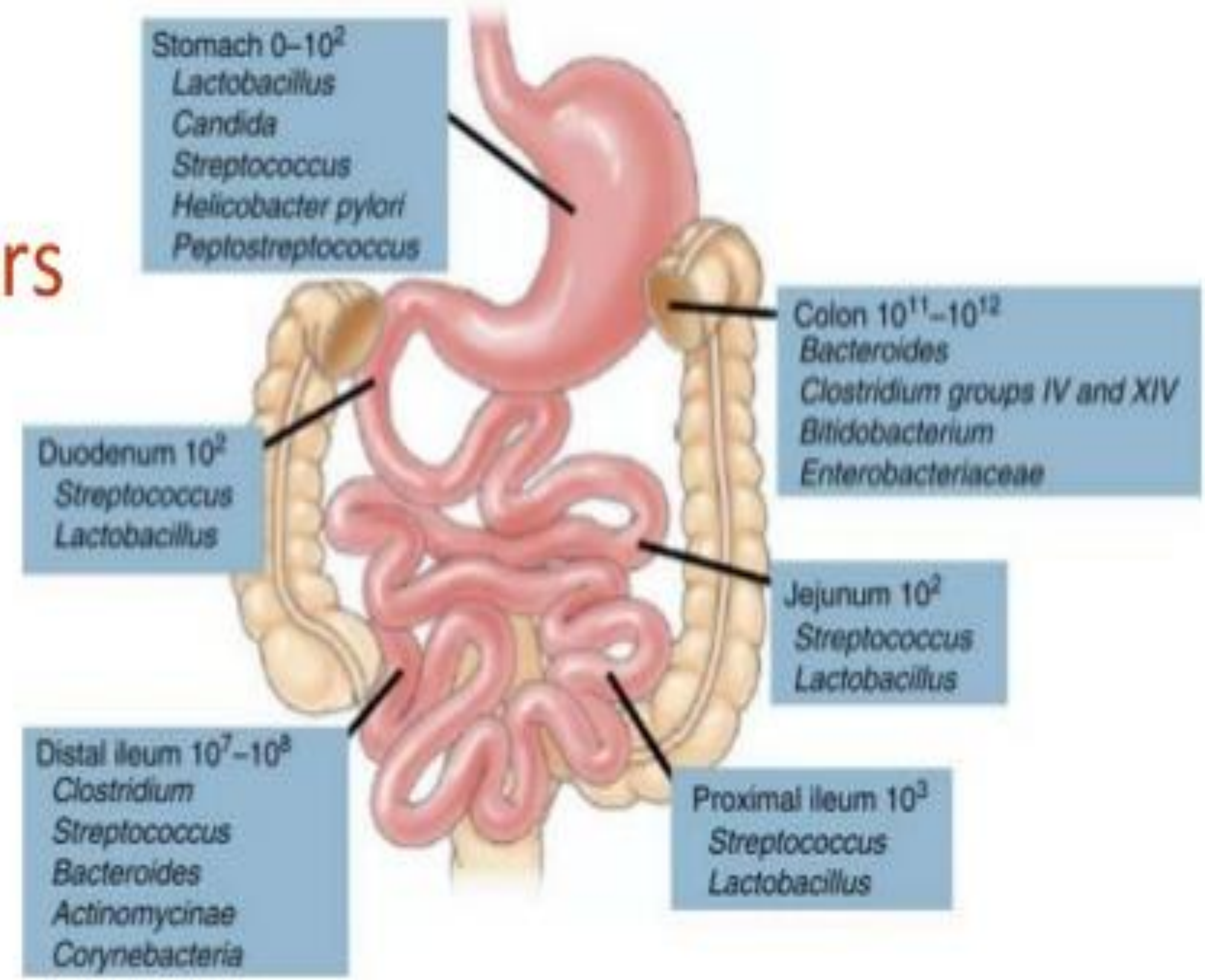
4. **Stimulate the development of certain tissues**
   i.e., intestines, certain lymphatic tissues, capillary density

5. **Stimulate the production of cross-reactive antibodies**.
   Low levels of antibodies produced against components of the normal flora are known to cross react with certain related pathogens, and thereby prevent infection or invasion.



a
Salivary gland
Oral cavity, 10^{8-9} cells/ml
Esophagus
Liver
Stomach
Duodenum
Pancreas
Colon
Ileocecal valve
Jejunum
Cecum
Ileum
Rectum
b
pH gradient
Stomach
1.5–5
Duodenum
5–7
Jejunum
7–9
Ileum
7–8
Colon
5–7
Microbial biomass
10^{2-3} cells/ml
10^{3-4} cells/ml
10^{4-5} cells/ml
10^{8} cells/ml
Ileocecal valve
10^{11}cells/ml

Location Matters



DOI: 10.1038/ajgsup.2012.4 @DrBonnie360

How did we get started?

First report of the composition of human body site…

Science

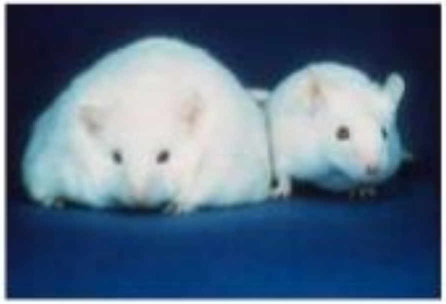
VOL 312 2 JUNE 2006

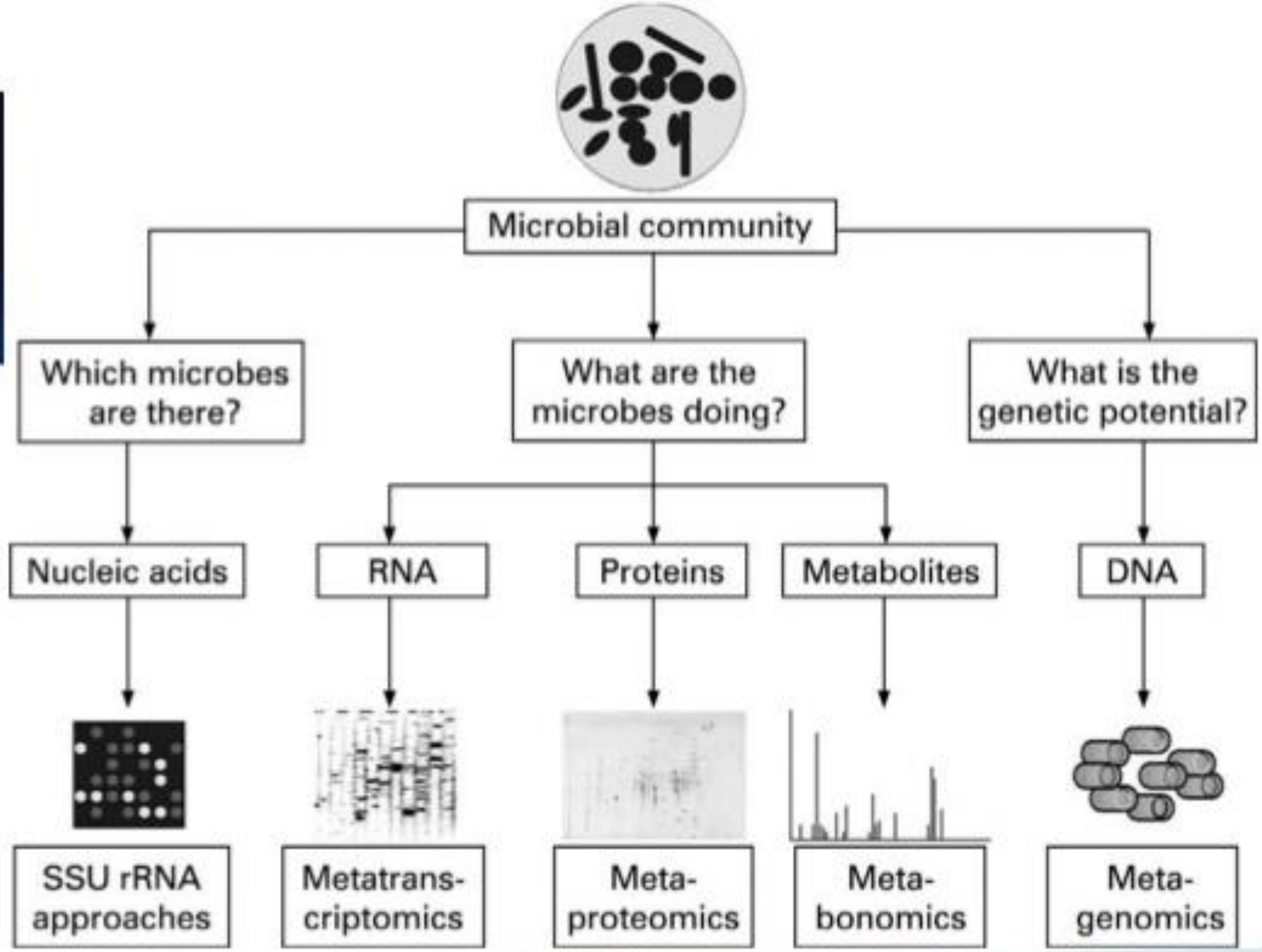
Metagenomic Analysis of the Human Distal Gut Microbiome

Steven R. Gill,[1*‡] Mihai Pop,[1†] Robert T. DeBoy,[1] Paul B. Eckburg,[2,3,4] Peter J. Turnbaugh,[5] Buck S. Samuel,[5] Jeffrey I. Gordon,[5] David A. Relman,[2,3,4] Claire M. Fraser-Liggett,[1,6] Karen E. Nelson[1]

If we can do one….can we do them all….

Metagenomics and other community-based "omics"



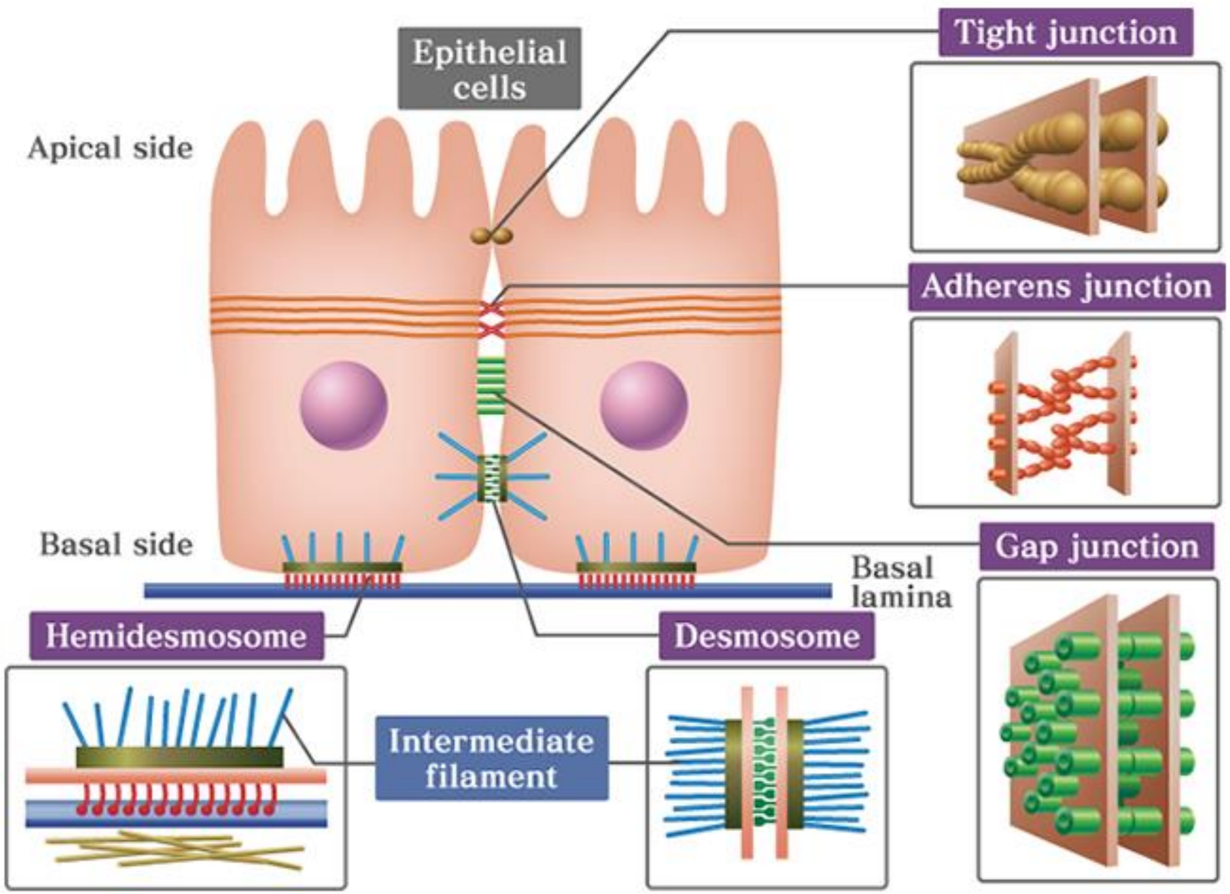


Zoetendal E G et al.
Gut 2008;57:1605-1615

International efforts to catalog the microbiome

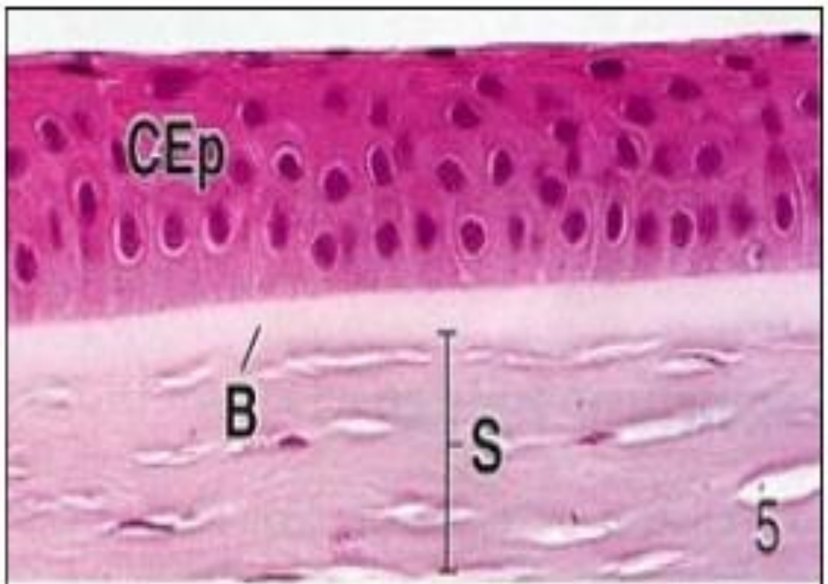
Ομοιόσταση μολυσμένου επιθυλιακού ιστού. Αναγεννητική φλεγμονή και βακτήρια.

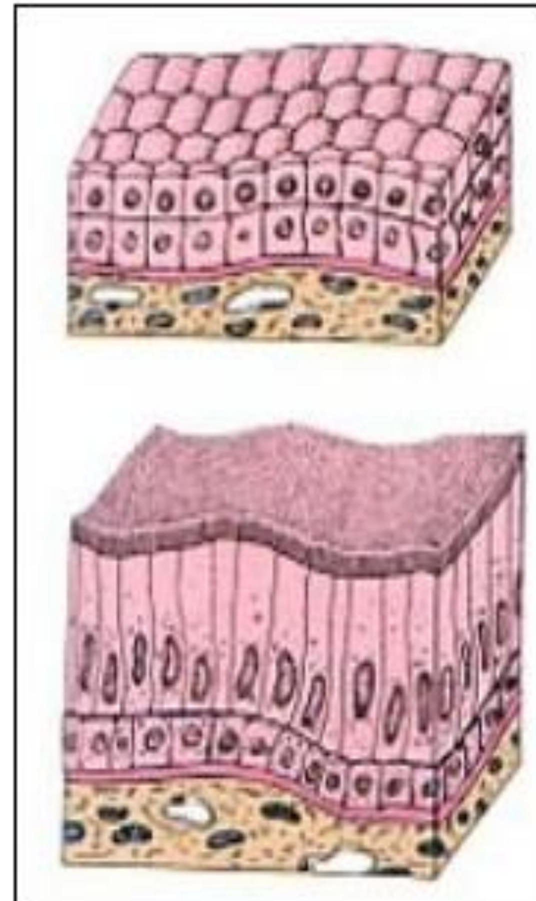
The epithelial layer



Epithelial tissue morphologic characteristics

- occupy boundary position
- consist of cells, lack extracellular substance
- rest on the basement membrane
- are polarized



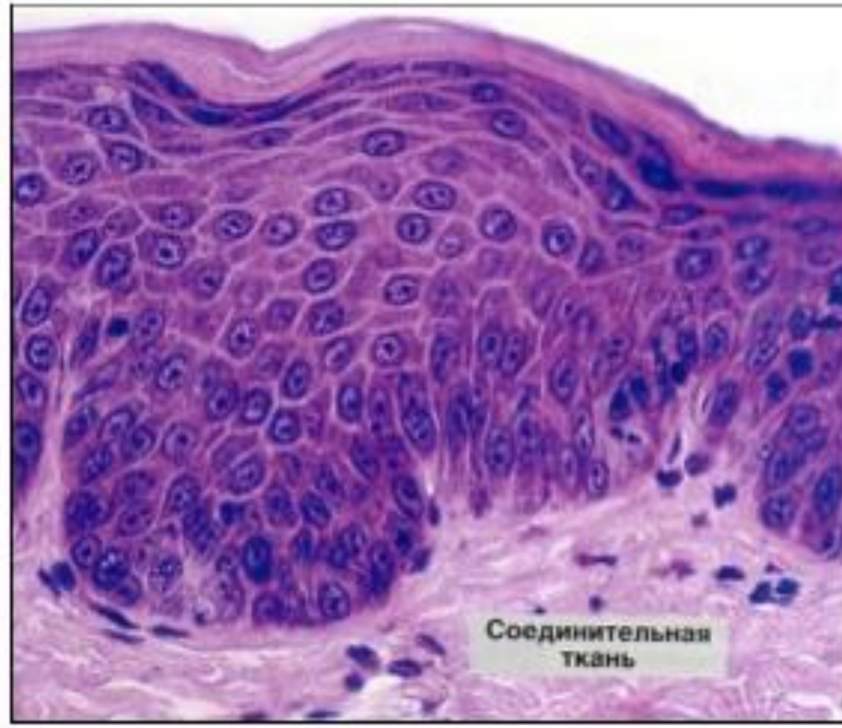


- are avascular
- possess a high capacity for regeneration

Thin skin epithelium

- - skin with hair follicles

⇔



Layers
- Stratum basale
- Stratum spinosum
- Stratum granulosum (poor-developed)
- Stratum corneum (thin)

innate immunity

έμφυτη ανοσία

adaptive immunity

προσαρμοστική ανοσία

Categories of Innate or Nonspecific Immunity

1. Differences in susceptibility to certain pathogens
2. Anatomical defense
3. Tissue bactericides, including complement
4. Microbial antagonism
5. Inflammation (ability to undergo an inflammatory response)
6. Phagocytosis

The first four categories are generally considered non cellular defenses.Inflammation and Phagocytosis are forms of cellular defense.

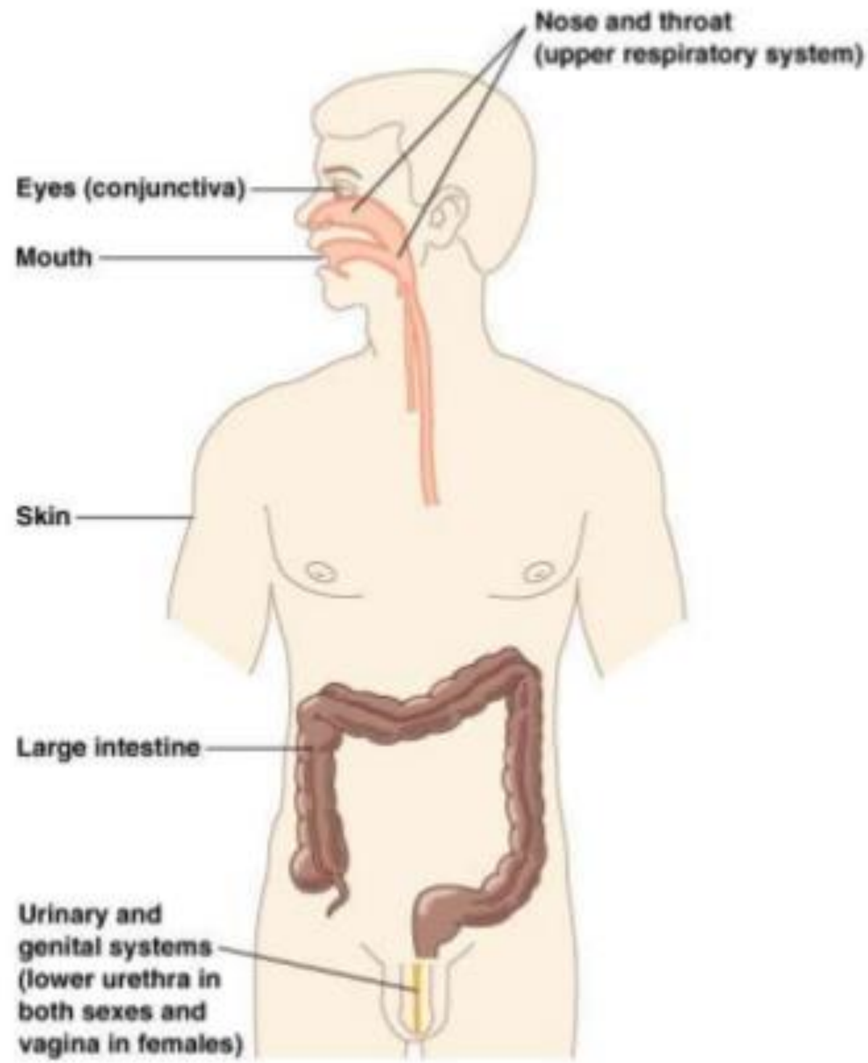
Microbial antagonism

There are three main ways that the normal flora protect the surfaces where they are colonized:

1) Competition with non-indigenous species for binding (colonization) sites.
2) Specific antagonism against non-indigenous species. Members of the normal flora may produce very specific proteins called bacteriocins which kill or inhibit other (usually closely-related) species of bacteria.
3) Nonspecific antagonism against non-indigenous species. The normal flora produce a variety of metabolites and end products that inhibit other microorganisms. These include fatty acids (lactate, propionate, etc.), peroxides and antibiotics.

Normal Microbiota and the Host:

- Locations of normal microbiota on and in the human body

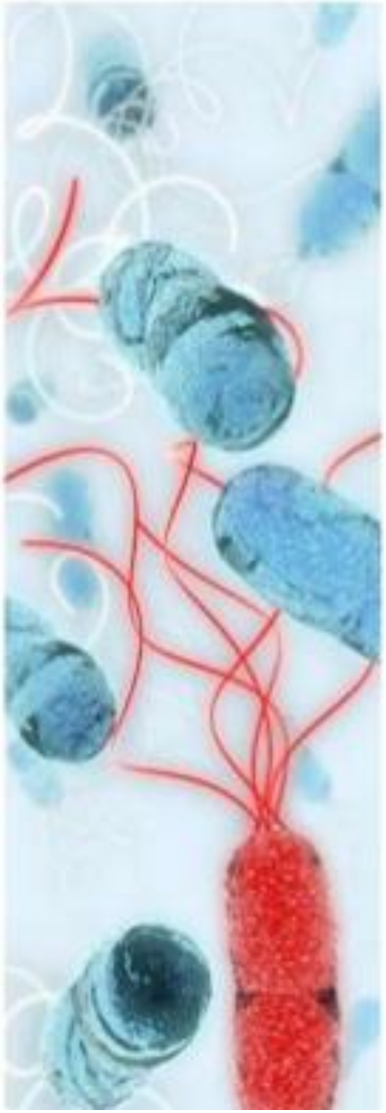


Normal Microbiota and the Host

- Transient microbiota may be present for days, weeks, or months

- Normal microbiota permanently colonize the host

- Symbiosis is the relationship between normal microbiota and the host



Dual necessity: Peacefully coexisting to achieve a mutually beneficial relationship.

- The host provides the microbiota a niche with a stable nutrient supply
- The microbiota performs essential functions for host physiology, including metabolic, digestive, and immune mechanisms
  - Regulate the host's metabolic function and energy balance
  - Provide the host with the capacity to hydrolyze complex plant sugars, synthesize vitamins, and detoxify xenobiotics in a mutualistic context
  - Affect the most fundamental of host physiological phenotypes, the rate of aging itself

Commensal gut microbiota protects the host from infection via both direct and indirect mechanisms.

The symbiosis between microbes and humans provides a stable and common metabolic pattern and well-balanced physiological homeostasis.

- CATEGORIES OF NORMAL FLORA

1. Symbionts (Mutualism)

   -- the microbe and the host are of benefit to one another

   -- ex. Normal flora of the large intestine

2. Commensals (Commensalism)

   --neutral relationship to the host

   -- microbe receives benefit from the host but host receives no harm/benefit from the microbe

   a. Resident Flora

   – most of the flora of the body; present invariably or for weeks/months in a particular environment

b. Transient Flora

- Non-pathogenic or potentially pathogenic microorganisms that establishes themselves briefly for colonization or infection without disease
- Ex. Neisseria

3. Opportunists

- potential pathogens
- lack the ability to invade and cause disease in healthy individuals

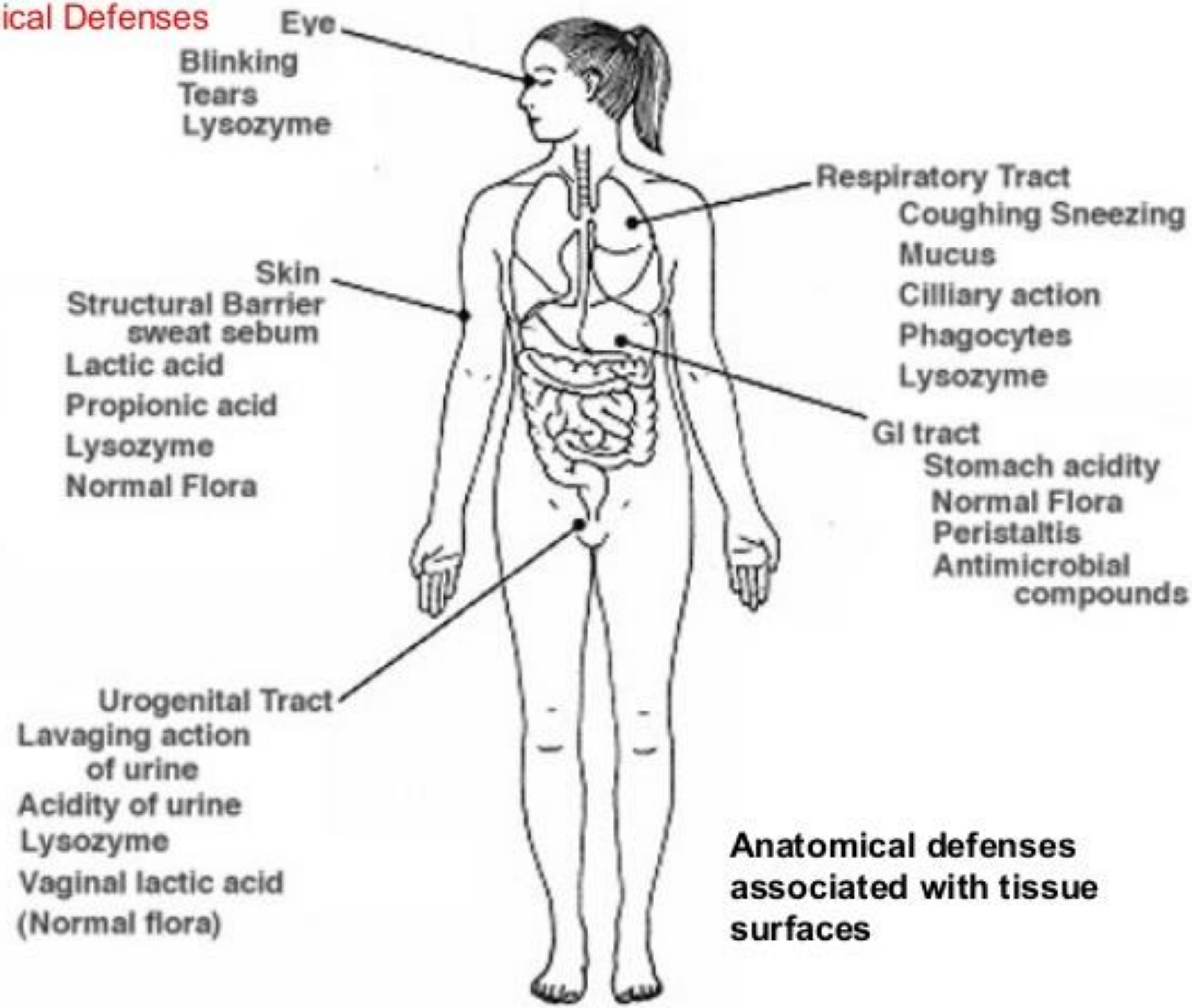
Normal Microbiota and the Host:

- Microbial antagonism is competition between microbes.

- Normal microbiota protect the host by:
  - occupying niches that pathogens might occupy
  - producing acids
  - producing bacteriocins

- Probiotics are live microbes applied to or ingested into the body, intended to exert a beneficial effect.

2. Anatomical Defenses



Anatomical defenses associated with tissue surfaces

Antimicrobial substances

Substance	Common Sources	Chemical Composition	Activity
Lysozyme	Serum, saliva, sweat, tears	Protein	Bacterial cell lysis
Complement	Serum	Protein-carbohydrate lipoprotein complex	Cell death or lysis of bacteria; participates in inflammation
histones, ß-lysins and other cationic proteins	Serum or organized tissues	Proteins or basic peptides	Disruption of bacterial plasma membrane
Lactoferrin and transferrin	Body secretions, serum, organized tissue spaces	Glycoprotein	Inhibit microbial growth by binding (withholding) iron
Peroxidase	Saliva, tissues, cells (neutrophils)	Protein	Act with peroxide to cause lethal oxidations of cells
Fibronectin	Serum and mucosal surfaces	Glycoprotein	Clearance of bacteria (opsonization)
Interferons	Virus-infected cells, lymphocytes	Protein	Resistance to virus infections
Interleukins	Macrophages, lymphocytes	Protein	Cause fever; promote activation of immune system

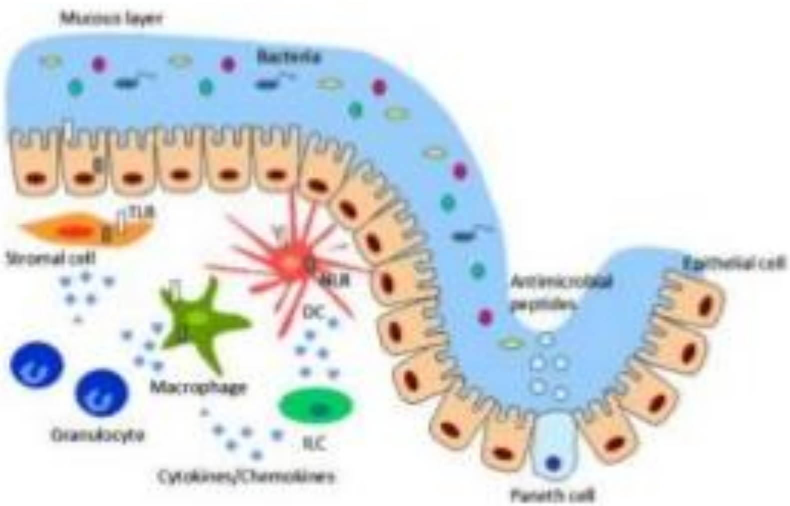
Anti-Microbial Peptides

- Made by neutrophils and some epithelial cells (small intestines, small airways)
- Short, cationic peptides (most 29-35 amino acids long)
- Interact strongly with acidic phospholipids and thought to form pores in membrane (eucaryotic membranes often have negative charge on carbohydrate rather than on phospholipid of outside of bilayer; may account for greater effect of peptides on microbes)
- Differentially active against different micro-organisms (evasion?)

Recognition of an infection once it gets past the epithelial barrier

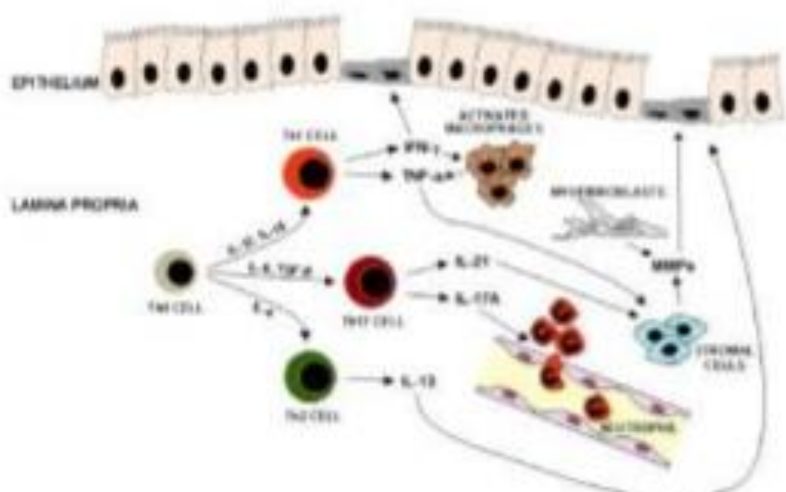
- <u>Soluble innate immune recognition elements</u> (collectins, ficolins, complement)
- <u>Sentinel innate immune cells of tissues</u>:

tissue macrophages, mast cells and immature dendritic cells, which induce inflammation (1st two) and trigger adaptive immunity (DCs)



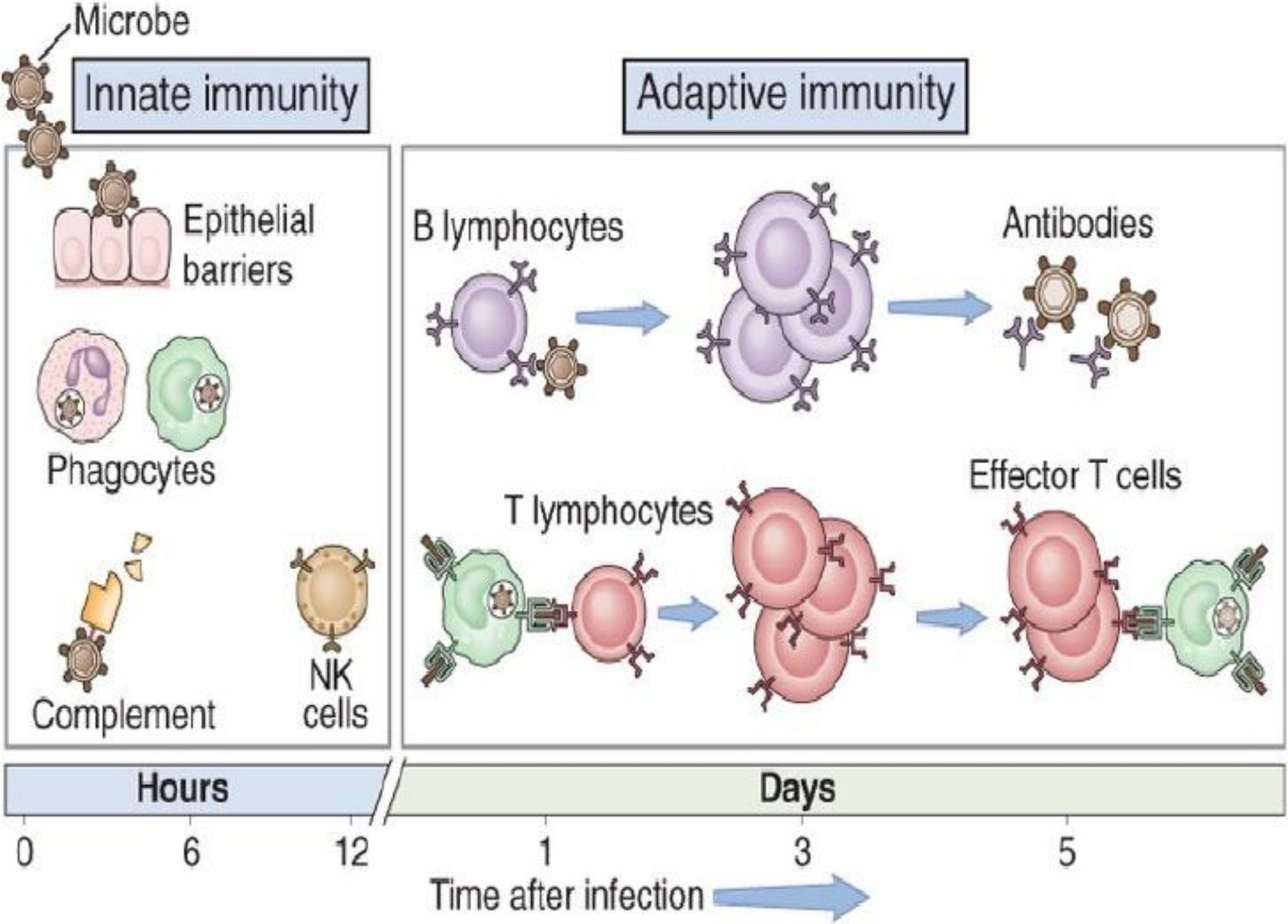
The innate immune system is the first line of defense against pathogens and is initiated by genome-encoded pattern recognition receptors (PRRs) that recognize PAMP

- Non-specific and does not confer long-lasting immunity (memory)
- **Immune cells:** dendritic cells (DCs) and macrophages, intestinal epithelial cells and myofibroblasts

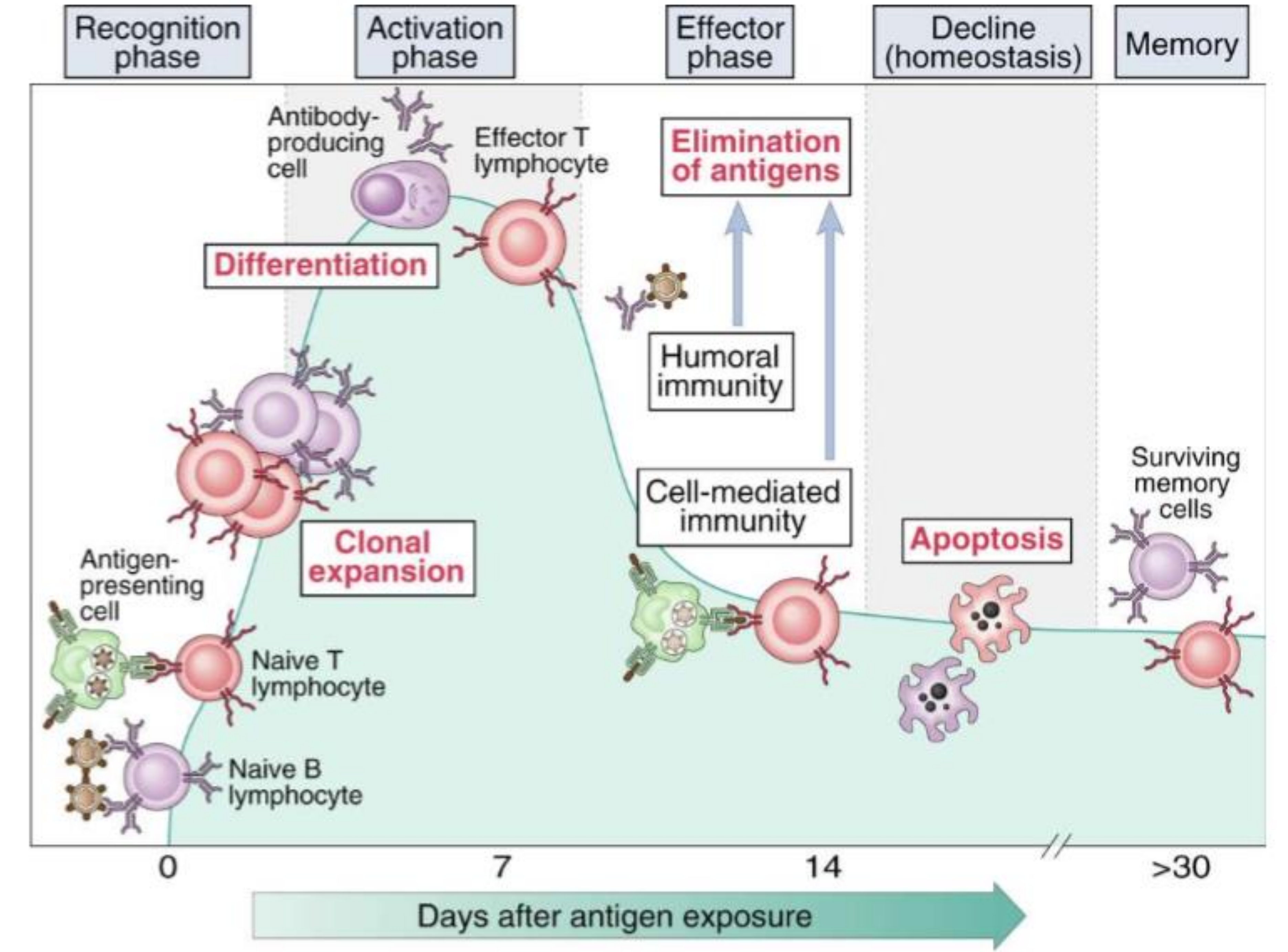


The adaptive immunity is highly specific, confers long lasting immunity, and is adaptable

- Cooperation between the molecules and cells of the innate immune system mounts an effective immune response
- **Key players:** T cells – Th1, Th2 or Th17 cell



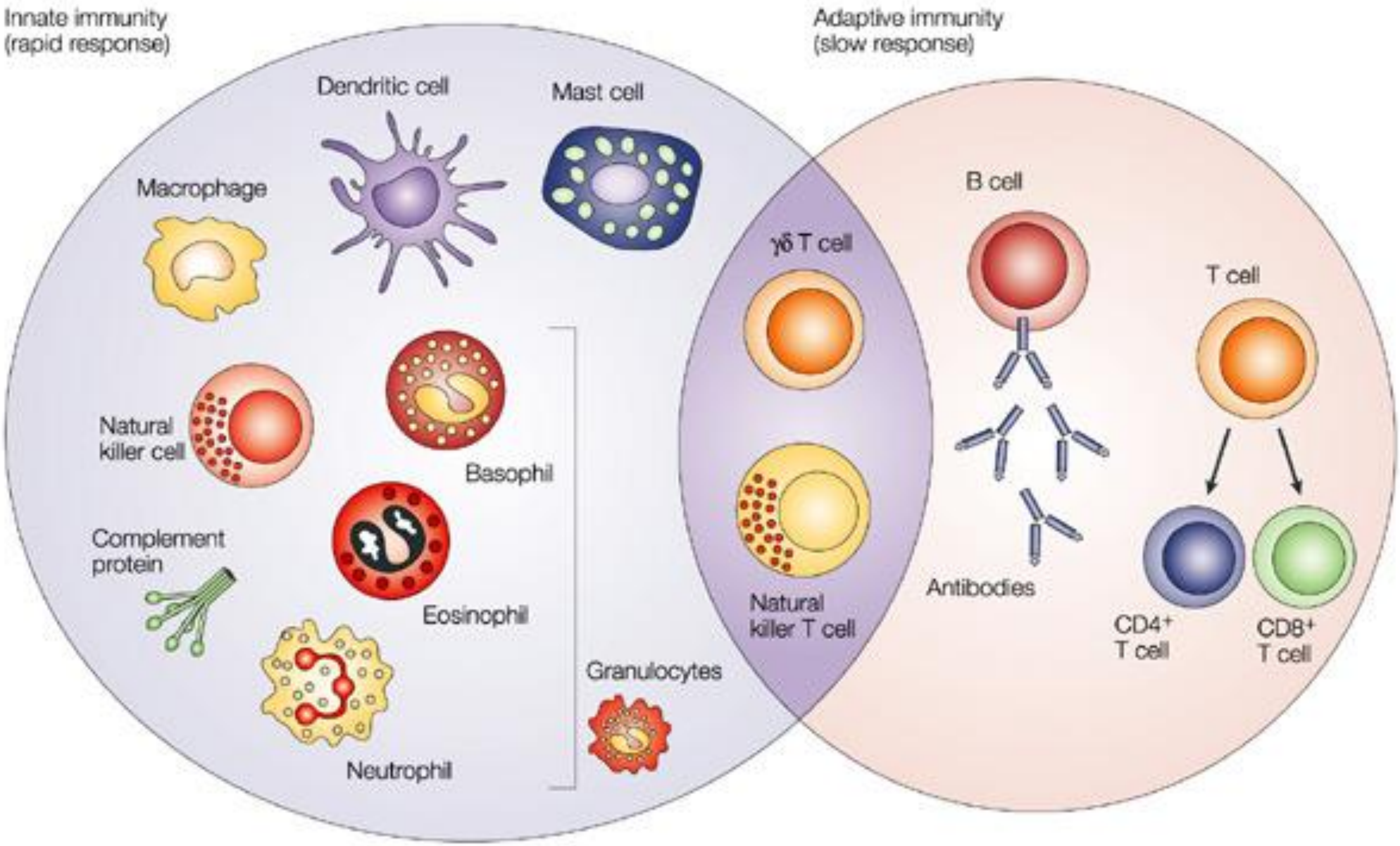
Microbe
Innate immunity
Epithelial barriers
Phagocytes
Complement
NK cells
Adaptive immunity
B lymphocytes
Antibodies
T lymphocytes
Effector T cells
Hours
Days
0
6
12
1
3
5
Time after infection



Recognition phase
Activation phase
Effector phase
Decline (homeostasis)
Memory
Antibody-producing cell
Effector T lymphocyte
Elimination of antigens
Differentiation
Humoral immunity
Cell-mediated immunity
Clonal expansion
Apoptosis
Surviving memory cells
Antigen-presenting cell
Naive T lymphocyte
Naive B lymphocyte
0
7
14
>30
Days after antigen exposure

Innate Immunity is a form of non specific host defense against invading bacteria. It is natural or “innate” to the host, depending, in part, on genetics. Innate defense mechanisms are constitutive to the host, meaning they are continually ready to respond to invasion and do not require a period of time for induction.

Innate Immune Response	Adaptive Immune Response
Immediate response, initiated within seconds	Gradual response, generated over 3-4 days
Targets groups of pathogens	Targets specific pathogens
No Memory	Memory



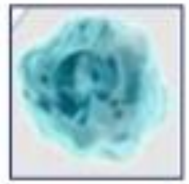
Nature Reviews | Cancer

Phagocytes and natural killer (NK) cells

Macrophages

- Long-lived phagocytes
- Mannose receptor, scavenger receptors
- Complement receptors
- ROS, RNI
- Produce proinflammatory cytokines (IL-1β, IL-12, TNF-α, IL-6,CXCL8)
- Produce growth factors for tissue remodeling
- Arise from circulating monocytes

Neutrophils



- Short-lived phagocytes
- Granules (acid hydrolase, myeloperoxidase, defensins, cathepsin G, lysozyme, lactoferrin, elastase, etc)
- Respiratory burst
- NETs (neutrophil extracellular traps: chromatin + serine proteases)
- Proinflammatory cytokines (IL-12, TNF-α)

NK cells

- Cytotoxic cells (via perforin and granzymes, or ADCC)
- Expansion/activation in response to IL-15, IL-12, Type I IFNs
- Produce IFN-γ, IL-1, and IL-2
- Recognize Class I MHC (inhibitory & activating receptors)
- Blood, spleen localization
- Memory?

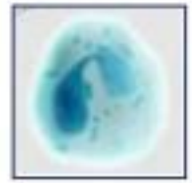
Mast cells

- Helminths, viruses and bacteria (also allergies)
- Respond to complement, IgE, TLR stimulation
- Release histamine, heparin, proteases, TNF and other proinflammatory cytokines, eicosanoids, etc.

Basophils

- Least abundant granulocyte
- Histamine, proteoglycans, lipid mediators, IL-4, IL-17E
- Macroparasite and helminth defense, allergy
- IgE-activated, matures via IL-3

Eosinophils



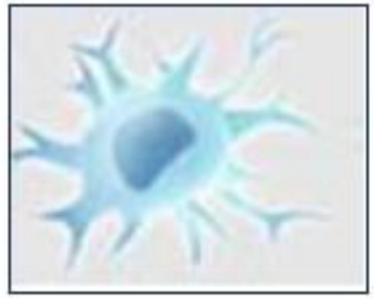
- Produce major basic protein, eicosanoids, histamine, peroxidases, acid phosphatase, growth factors, and cytokines
- Helminths, viral infections, allergies
- Activated by IL-5, IL-3, and GM-CSF

Nuocytes

- Important in Type 2 innate response against helminth infection[1]
- Respond to IL-25 and IL-33, produce IL-13
- Involved in allergic lung inflammation

(1) Neill, D.R. et al. (2010) Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature **464**, 1367-1370

Dendritic cells



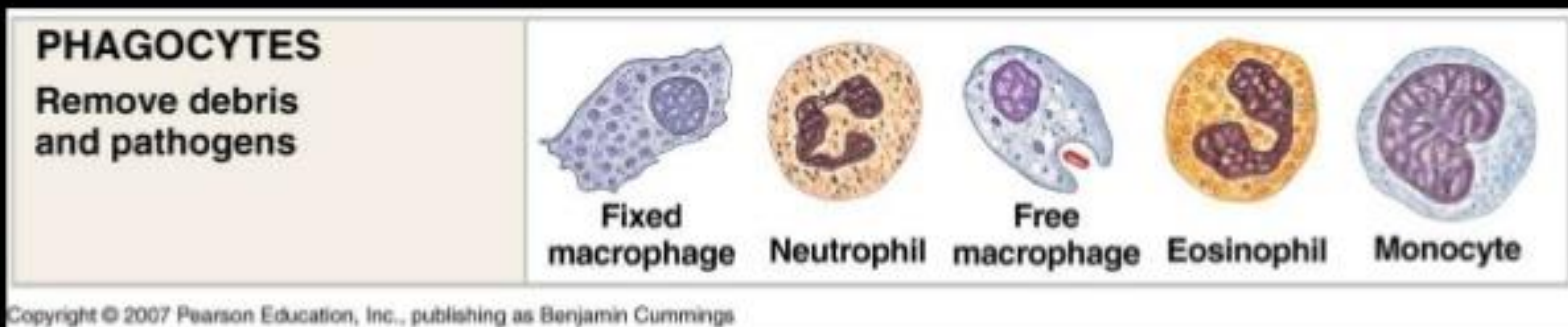
- "Professional antigen presenting cells (APCs)" – link innate and adaptive immunity (macrophages and B cells are also considered professional APCs)

- Present as immature DCs (highly phagocytic) in tissues; upon antigen capture, migrate to lymph nodes and spleen and mature, providing Ag presentation and co-stimulation to T cells

- Multiple subsets and sub-subsets based on surface markers, localization and function

- Produce IL-12, IL-15, TNF-α and Type I IFNs, and respond to chemokines through CCR2, CCR5, CCR6 and CCR7

Phagocytes: 1st Line of Cellular Defense

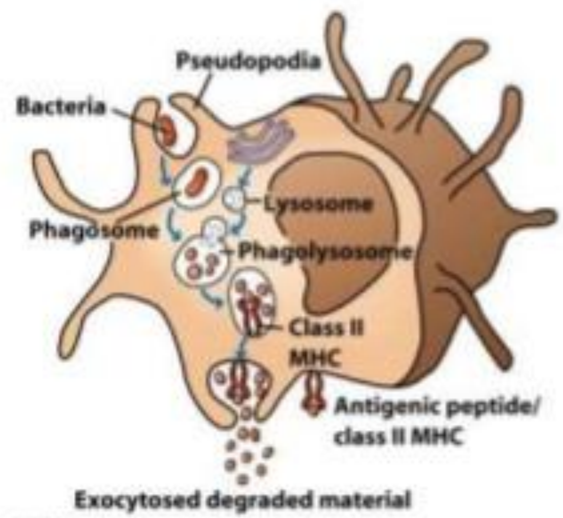


Microphages – neutrophils & eosinophils

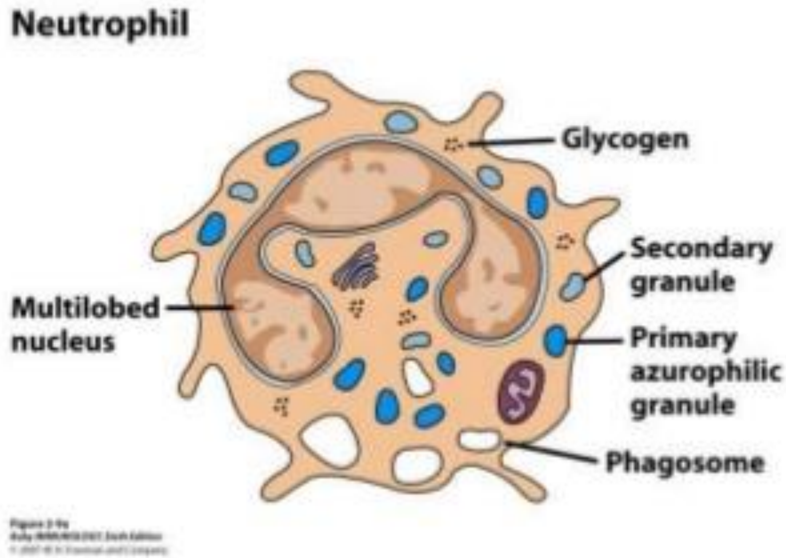
Macrophages – derived from monocytes

Phagocytes – 1) macrophages

- Ingest and digest whole microbes and present Ag to helper T cells
- CD14



Phagocytes – 2) neutrophils



- first to arrive at site of injury playing a key role in the front-line defense against invading pathogens

- express & release cytokines: amplify inflammatory reactions by other cells

- release soluble anti-microbials

Phagocytes – 3) dendritic cells

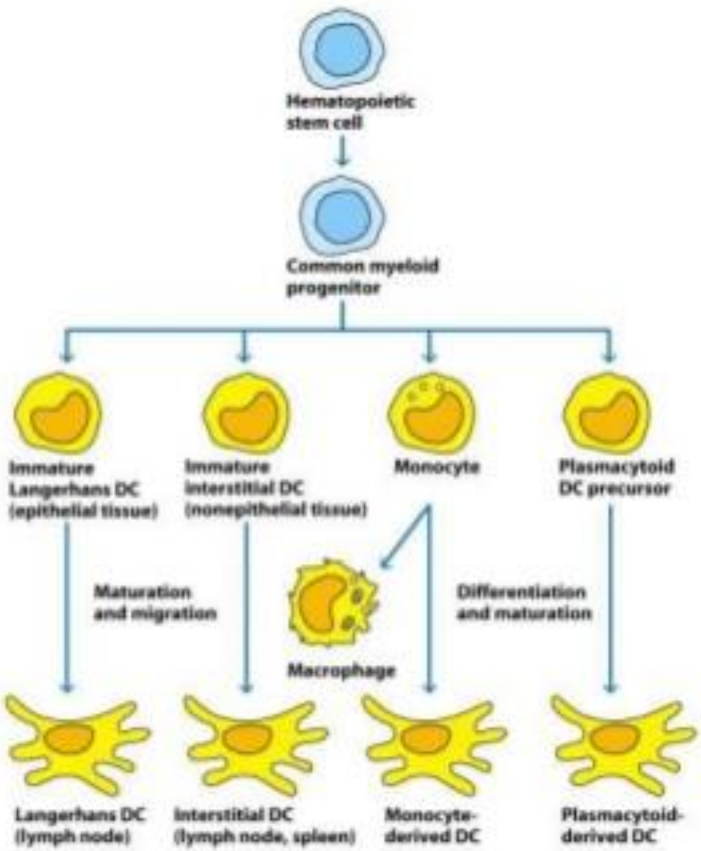
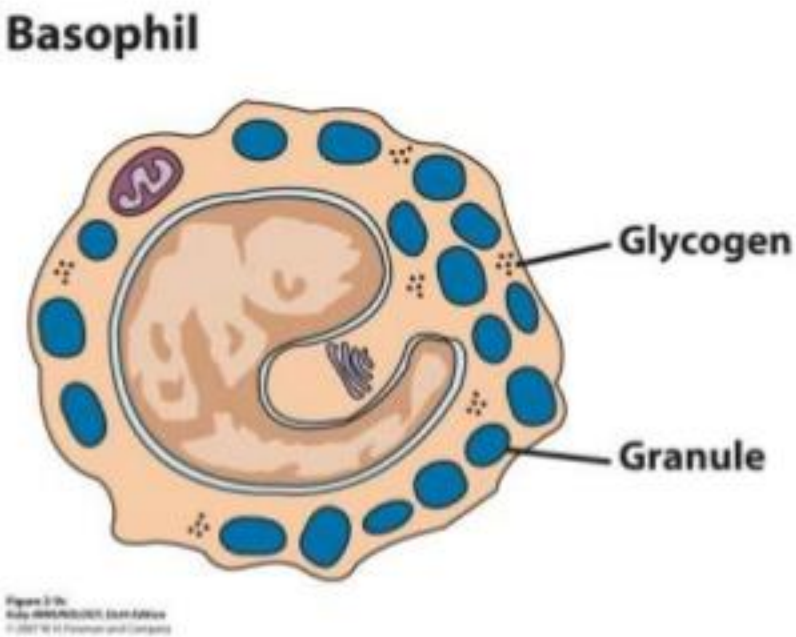


Figure 2-10
Kuby IMMUNOLOGY, Sixth Edition

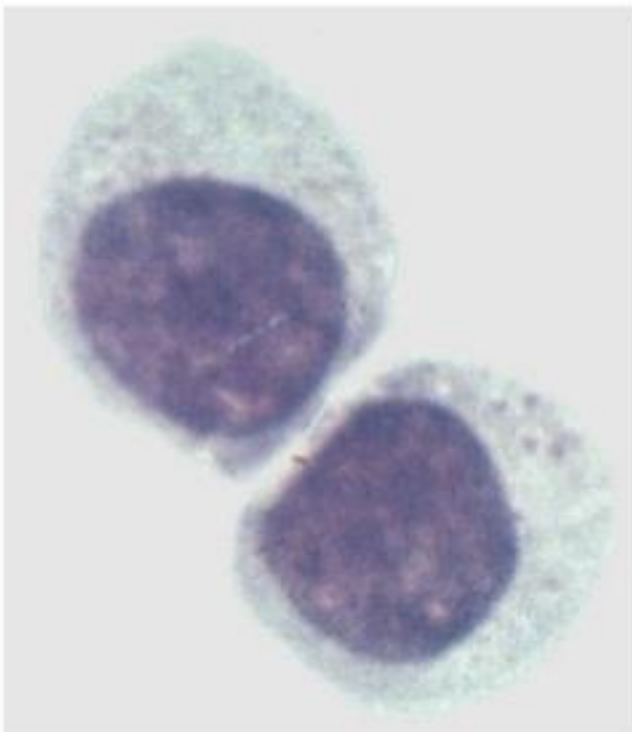
- Are complicated
- Excellent phagocytes
- Antigen-presenting cells

Basophils and Mast cells

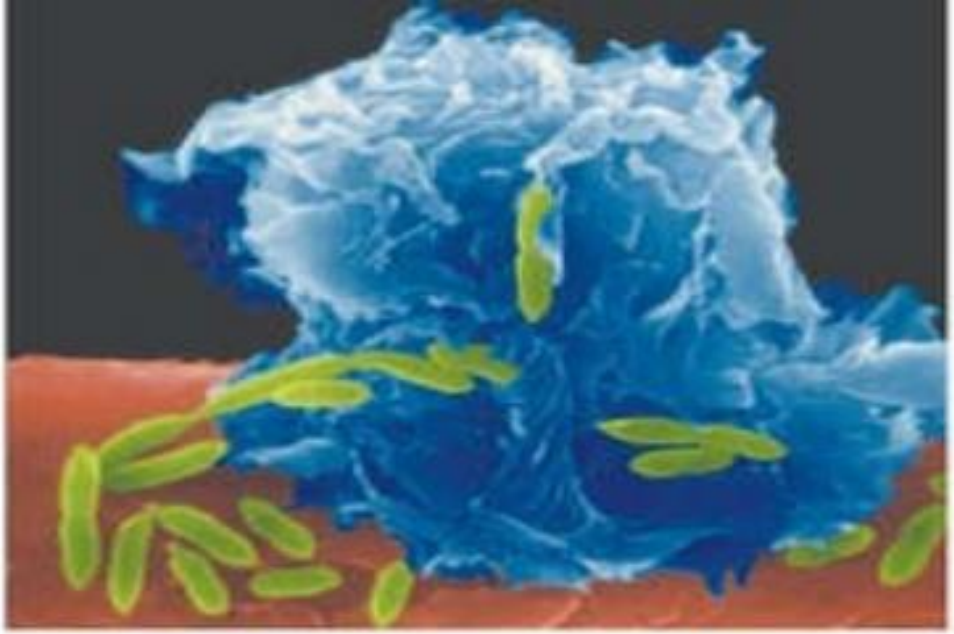


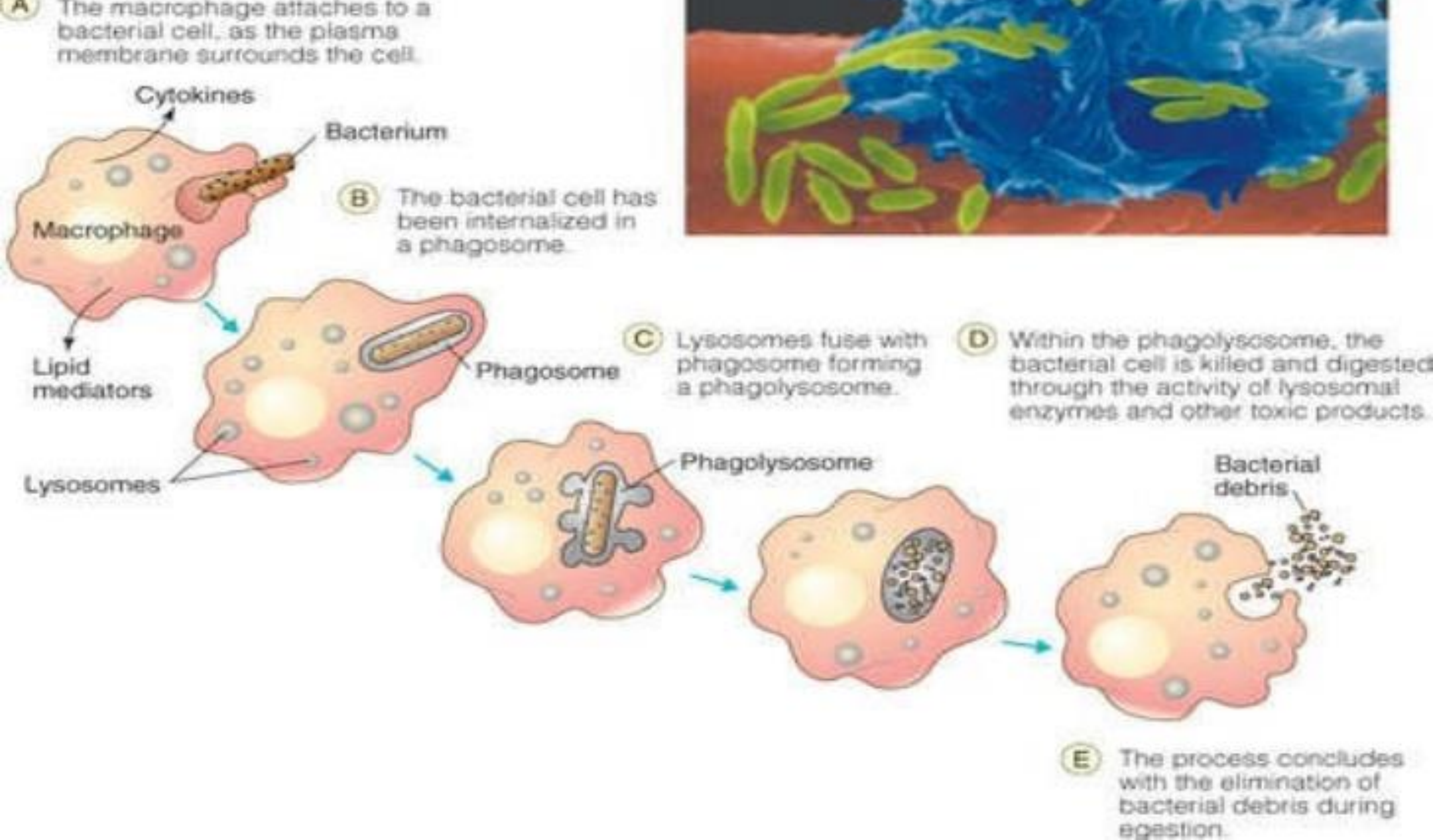
- Allergies
- Mast cells are anti-parasites
- Mast cells are minor phagocytes

Natural killer (NK) cells



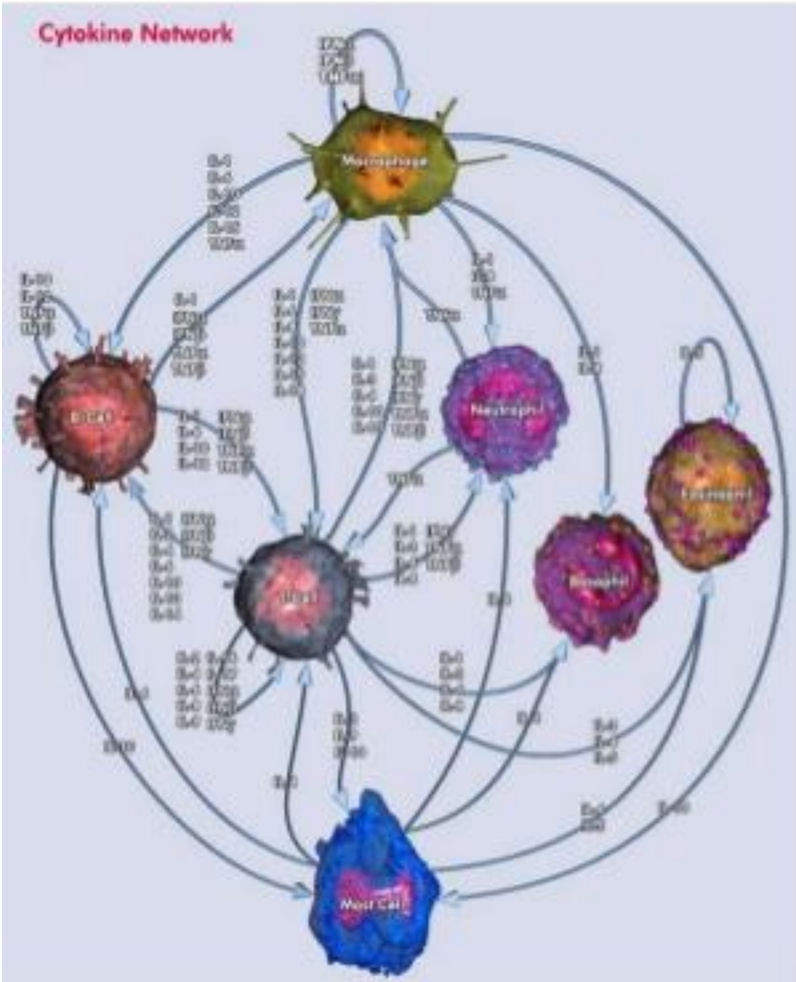
- large granular lymphocytes
- Kill virus-infected or transformed cells (no Ag recognition receptors)
- Cytotoxicity mechanisms not well understood





A The macrophage attaches to a bacterial cell, as the plasma membrane surrounds the cell.
Cytokines
Bacterium
Macrophage
Lipid mediators
Lysosomes
B The bacterial cell has been internalized in a phagosome.
Phagosome
C Lysosomes fuse with phagosome forming a phagolysosome.
Phagolysosome
D Within the phagolysosome, the bacterial cell is killed and digested through the activity of lysosomal enzymes and other toxic products.
Bacterial debris
E The process concludes with the elimination of bacterial debris during egestion.

Cytokines and chemokines



Cytokines produced by innate immune cells:

- Type I IFNs, IFN-g
- TNF-a
- IL-1, 6, 8, 10, 12, 15, 18

Cytokines that enhance/inhibit innate immune activity:

- Enhance: IFN-g, TNF-a, IL-15, IL-12
- Inhibit: IL-10, TGF-beta

Important chemokine receptors:

- CXCR1, 2 (neutrophils)
- CCR1, 2, 3 (leukocytes)
- CCR5 (DCs, NK cells, monocytes)
- CCR6, 7 (DCs)
- CXCR3 (ligand: CXCL10, monocytes and NK cells)

Important chemokines:

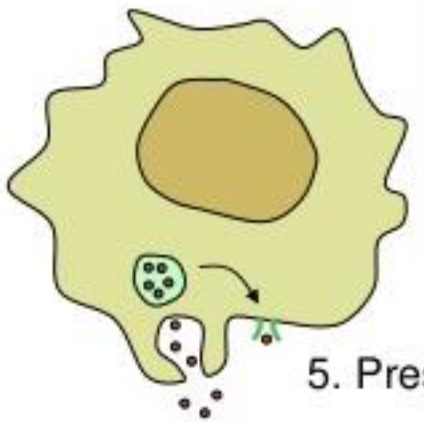
- CXCL8 (produced by M∅, recruits PMN)
- CCL2 (produced by monocytes and fibroblasts, and recruits monocytes, DCs, NK cells)

Phagocytosis (IV)

Functions

Essential for fighting infections and for subsequent immunity.

One liter of human blood contains about six billion phagocytes.



5. Present part of the degraded peptide to T cells.

6. Exocytose degraded material

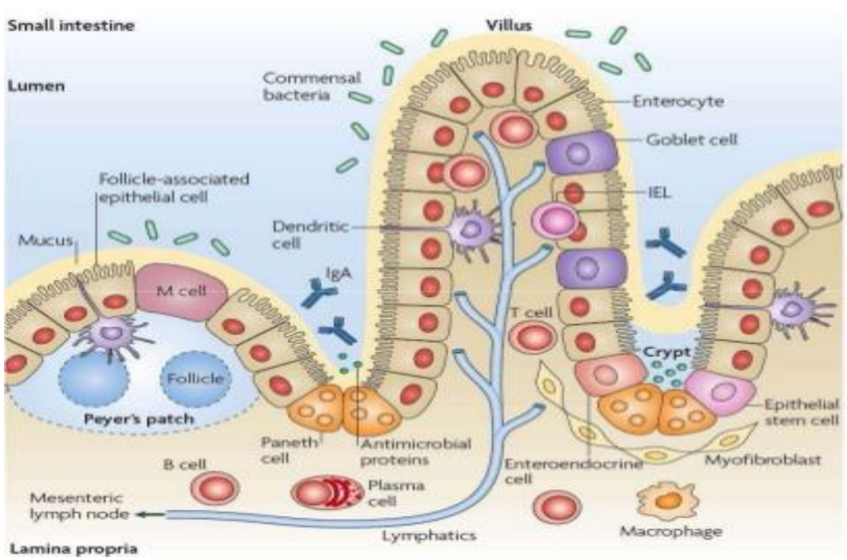
- **Innate immunity-**Immune mechanisms that are used by the host to immediately defend itself
- Comprises of bariers, complements, antimicrobial peptides, cytokines, macrophages, DCs , NK cells, PMNs

- **Dendritic cells** (DCs) are component of innate immune system. Their main function is to process antigen material and present it on the surface to T-cells, thus functioning as antigen-presenting cells.

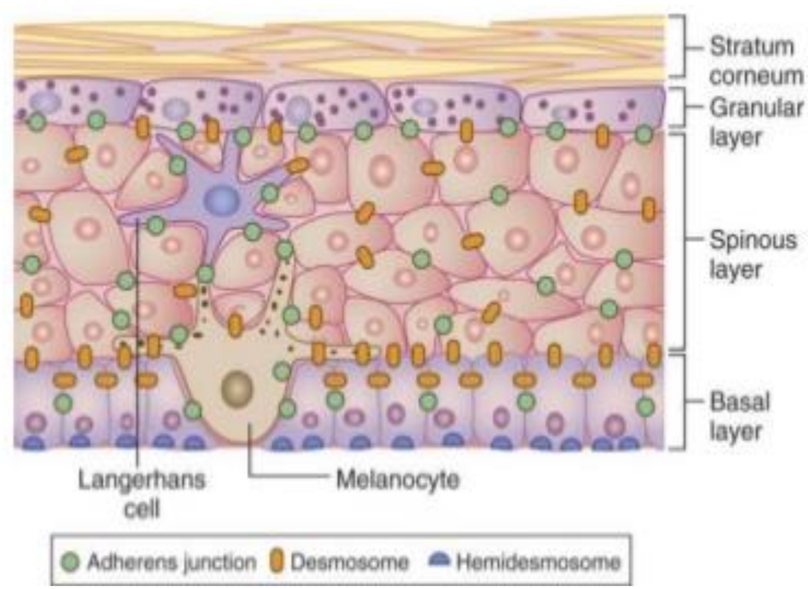
Antigen Presenting cells/ Dendritic cells

Dendritic cells are present in those tissues that are in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called the Langerhans cell) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood.

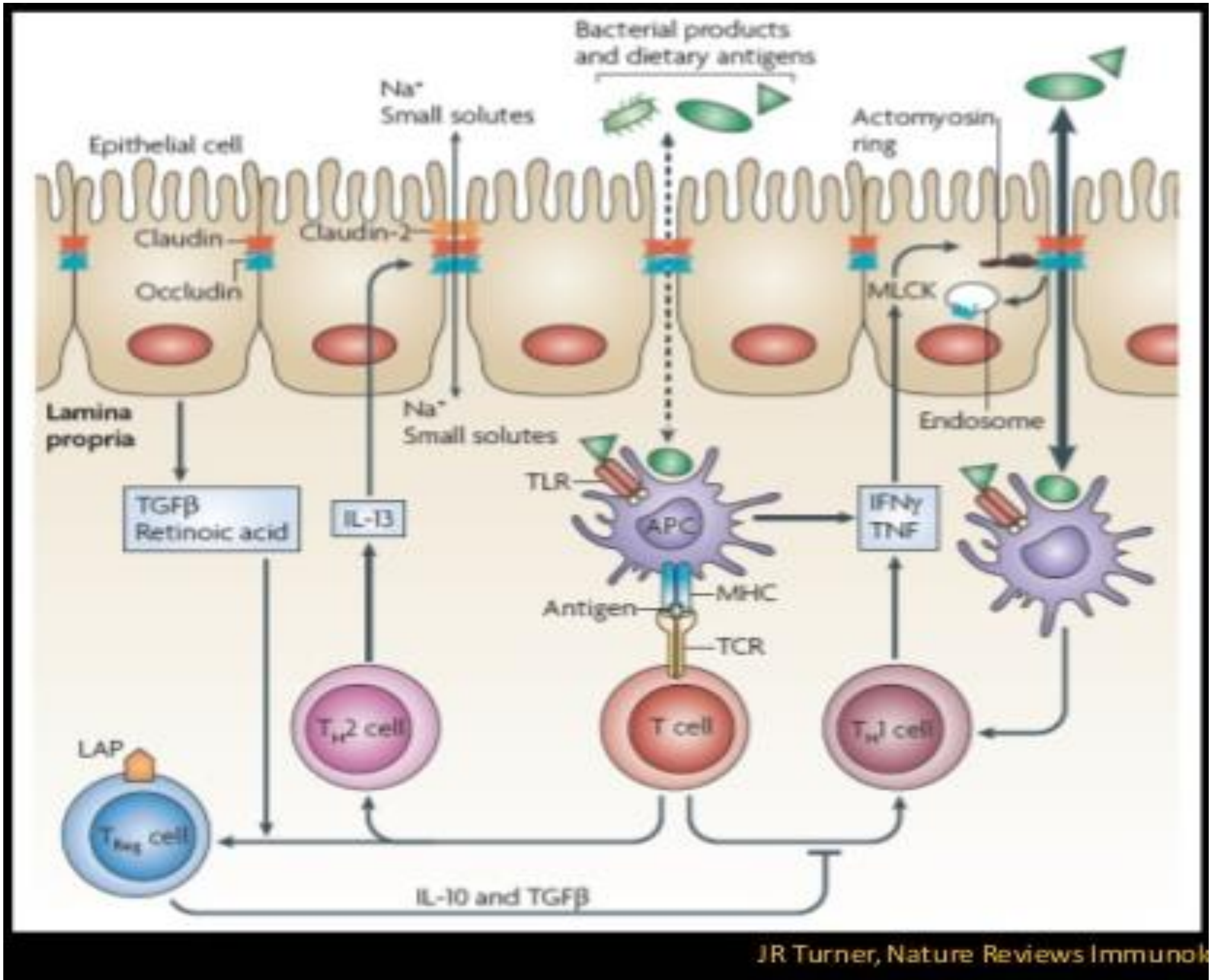
INTESTINAL EPITHELIUM



SKIN



Antigen Presenting cells/ Dendritic cells



Dendritic cells can extend processes across the epithelial layer to capture antigen from the lumen of the gut

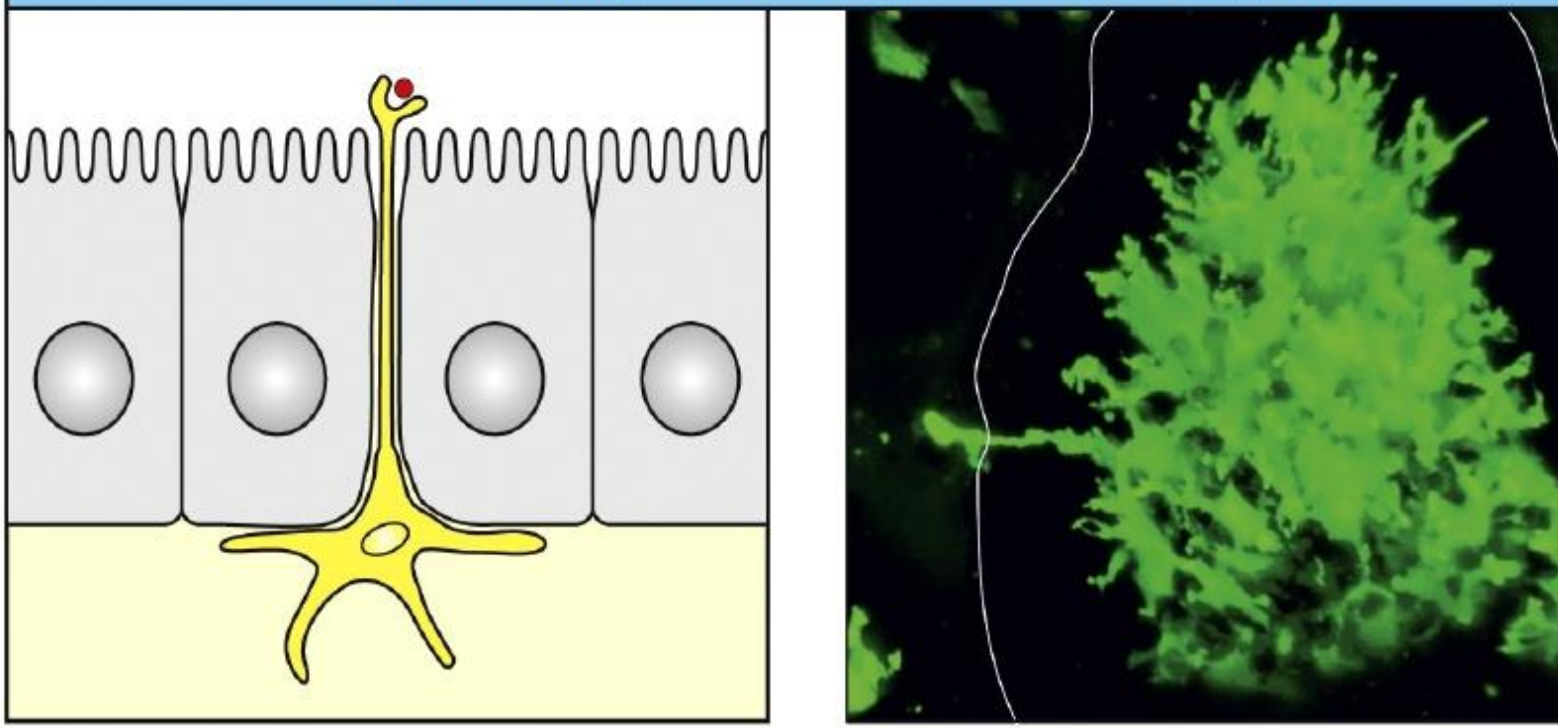


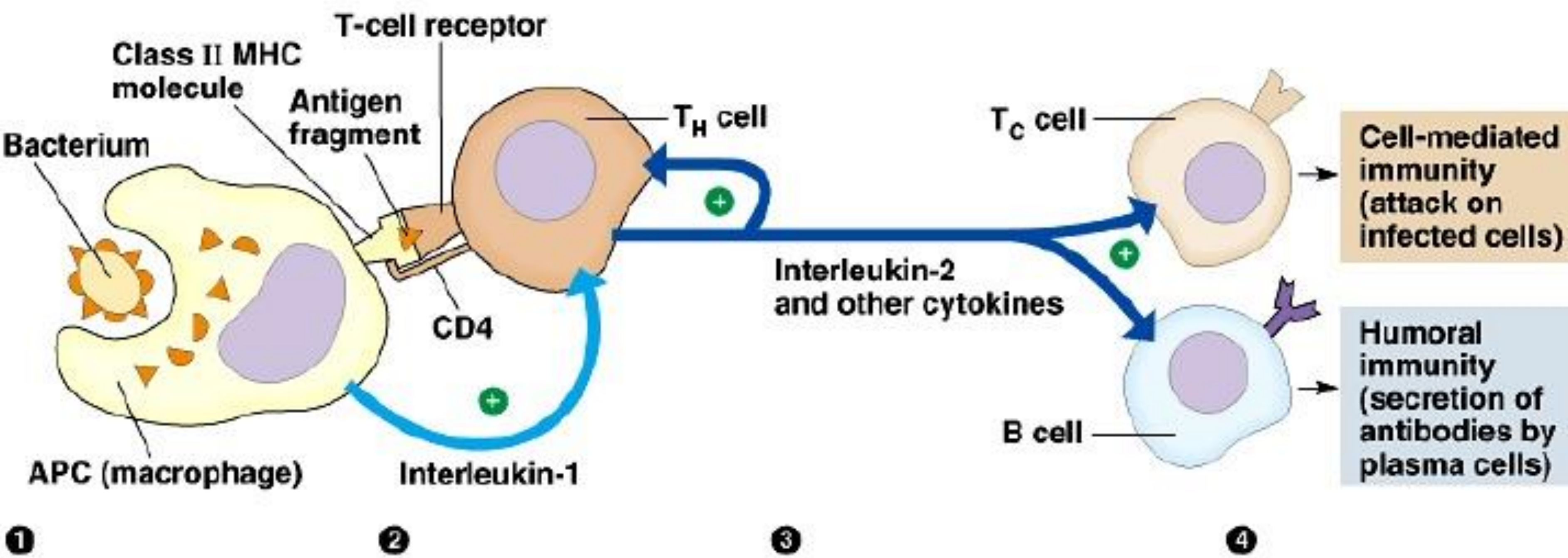
Figure 11-9 Immunobiology, 7ed. (© Garland Science 2008)

Dendritic cells recognize pathogens through pattern recognition receptors (PRRs):

- TLRs (LPS, peptidoglycan, unmethylated CpG motifs, double-stranded viral RNA)
  - TLR2 - Gram positive cell wall components
  - TLR4 - LPS from *E coli* - essential for maturation & cytokine production in LPS-stimulated murine DC
  - TLR5 - Flagellin from Gram negative bacteria
  - TLR9 - CpG motifs from bacterial DNA
- Mannose receptors
- NOD1
  - recognizes muramyl-tripeptides from Gram negative bacteria)
- NOD2
  - recognizes muramyl-dipeptides common to all peptidoglycans of all bacteria species)

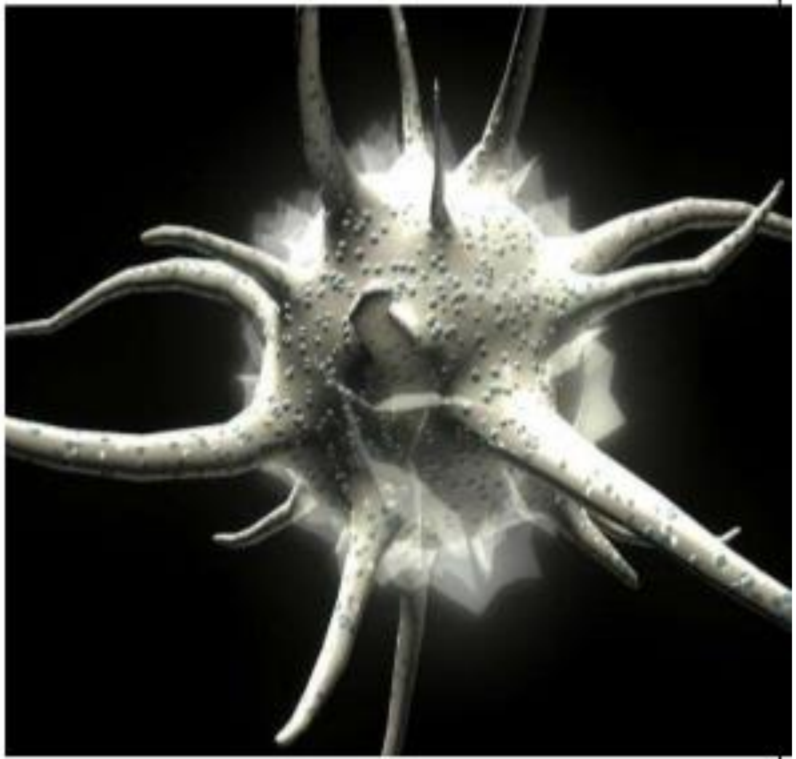
Helper T lymphocytes

- Function in both humoral & cell-mediated immunity
- Stimulated by antigen presenting cells (APCs)
- T cell surface protein CD4 enhances activation
- Cytokines secreted (stimulate other lymphocytes):
  a) interleukin-2 (IL-2): activates B cells and cytotoxic T cells
  b) interleukin-1 (IL-1): activates helper T cell to produce IL-2

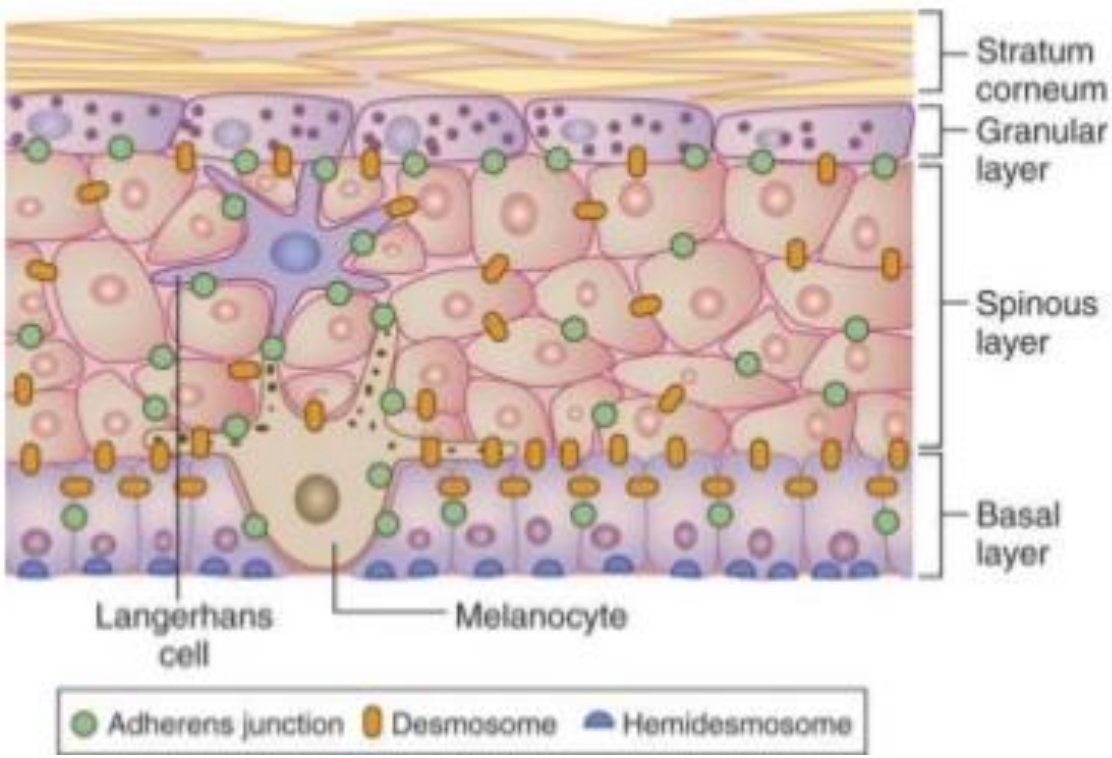


TYPES OF DENDRITIC CELLS

- Langerhans cells
- Dermal dendritic cells
- Melanocytes
- Merkel cells

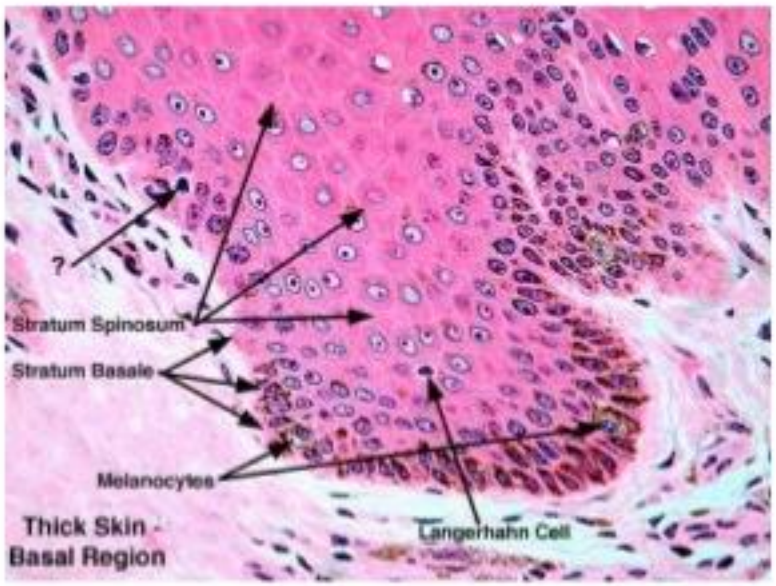


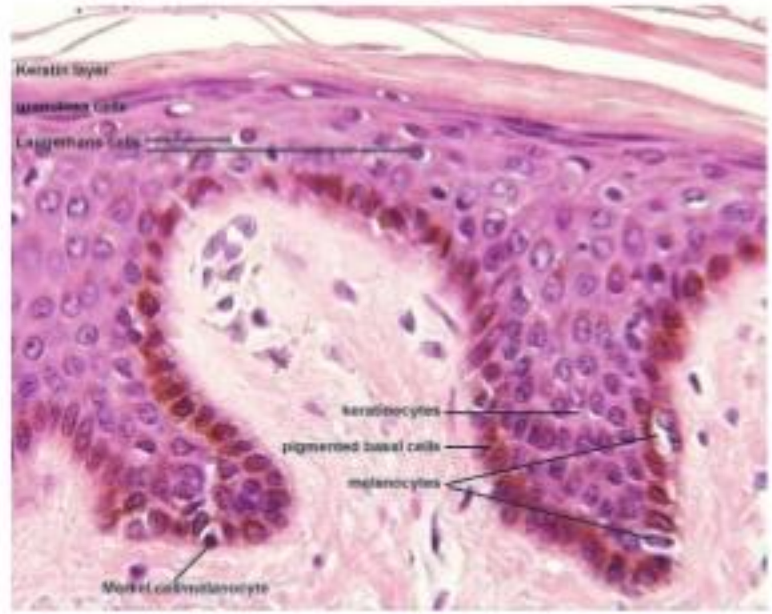
Dendritic cells are present in those tissues that are in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called the Langerhans cell) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood.



Histology

- Light microscopy : pale staining & convoluted nuclei





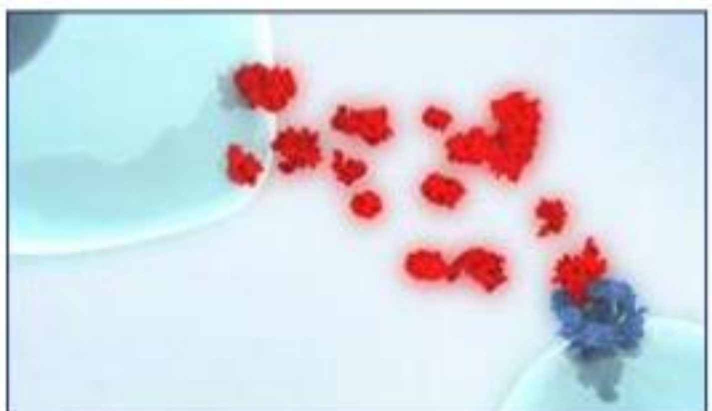
Outcomes of an innate immune response

Microbial killing

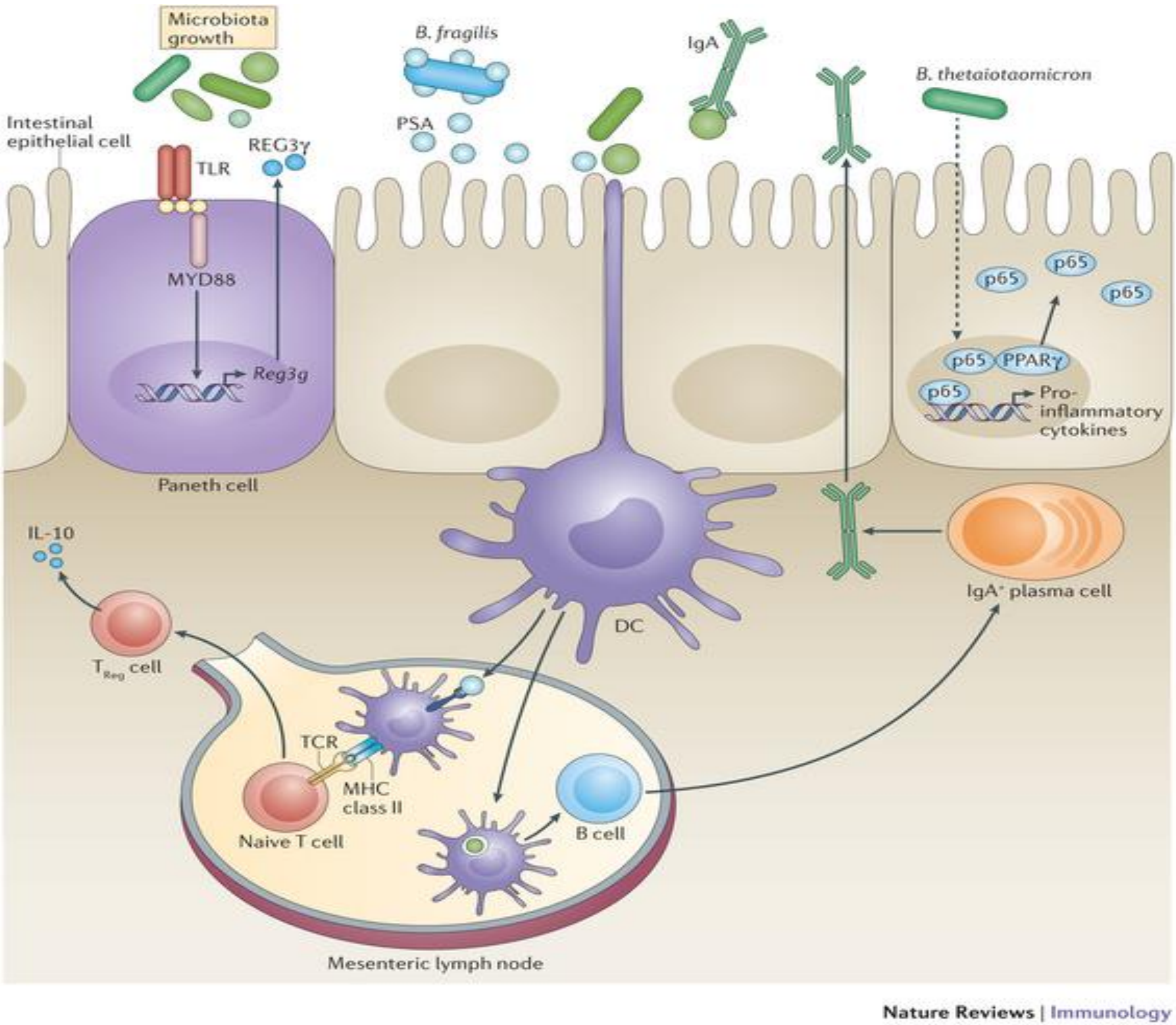
- Complement
- Neutrophil and macrophage microbicidal mechanisms
- NK cell cytotoxicity against infected cells
- Type I IFNs induce antiviral state in infected cells

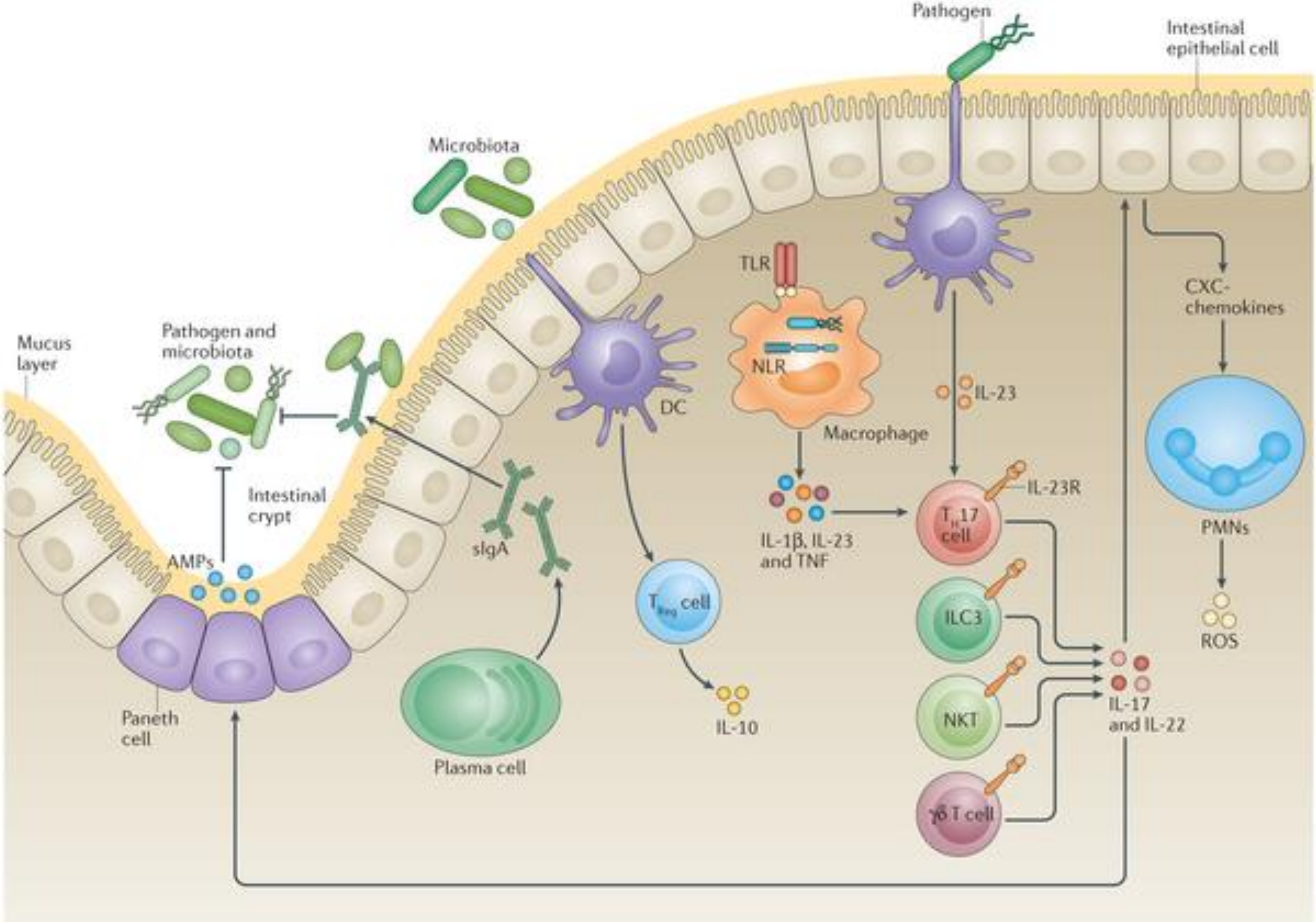
Induction of adaptive immunity

- Dendritic cells and macrophages present antigens to T cells and provide co-stimulation
- Chemokine and cytokine production leads to lymphocyte expansion and activation

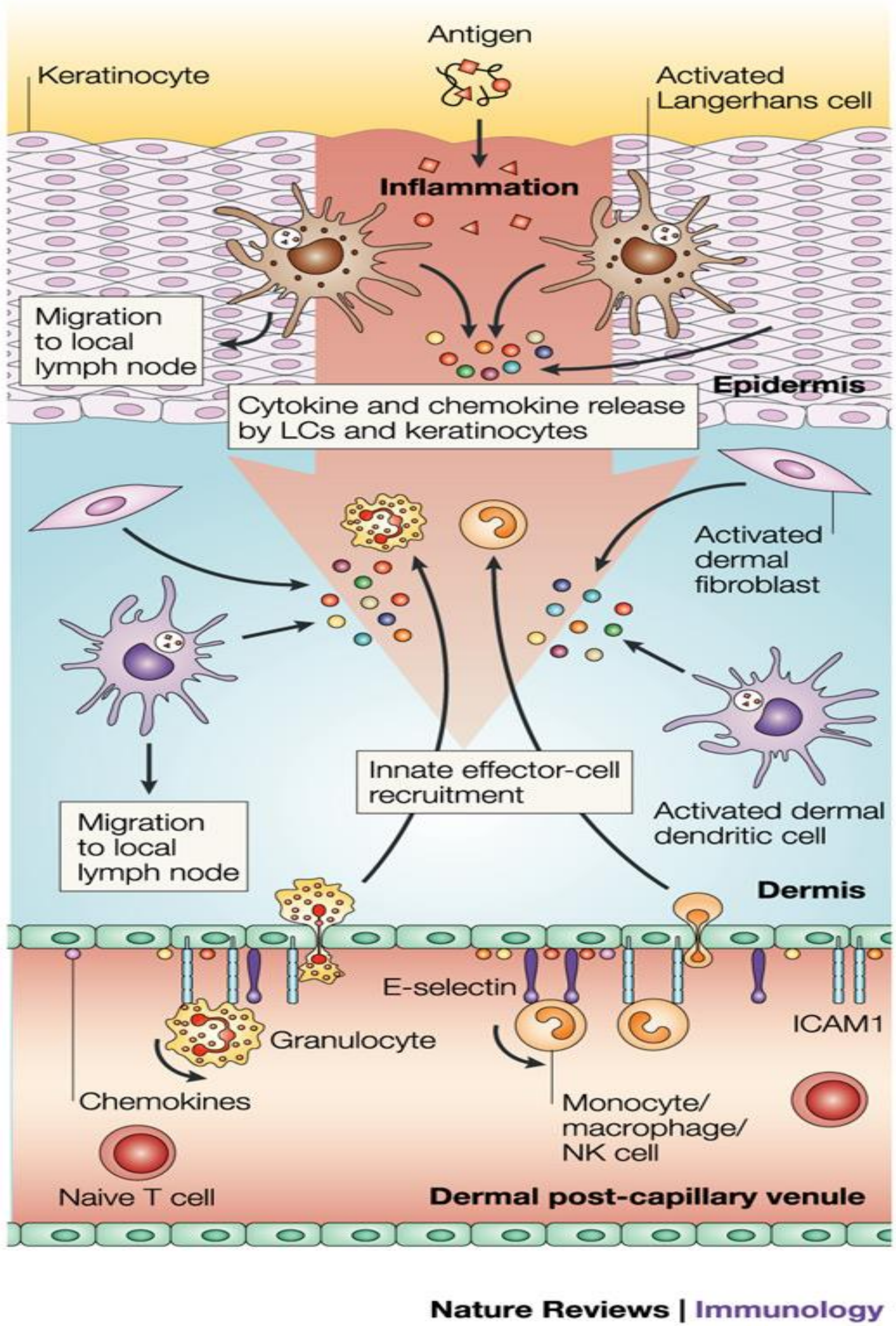


Mucosal immunity



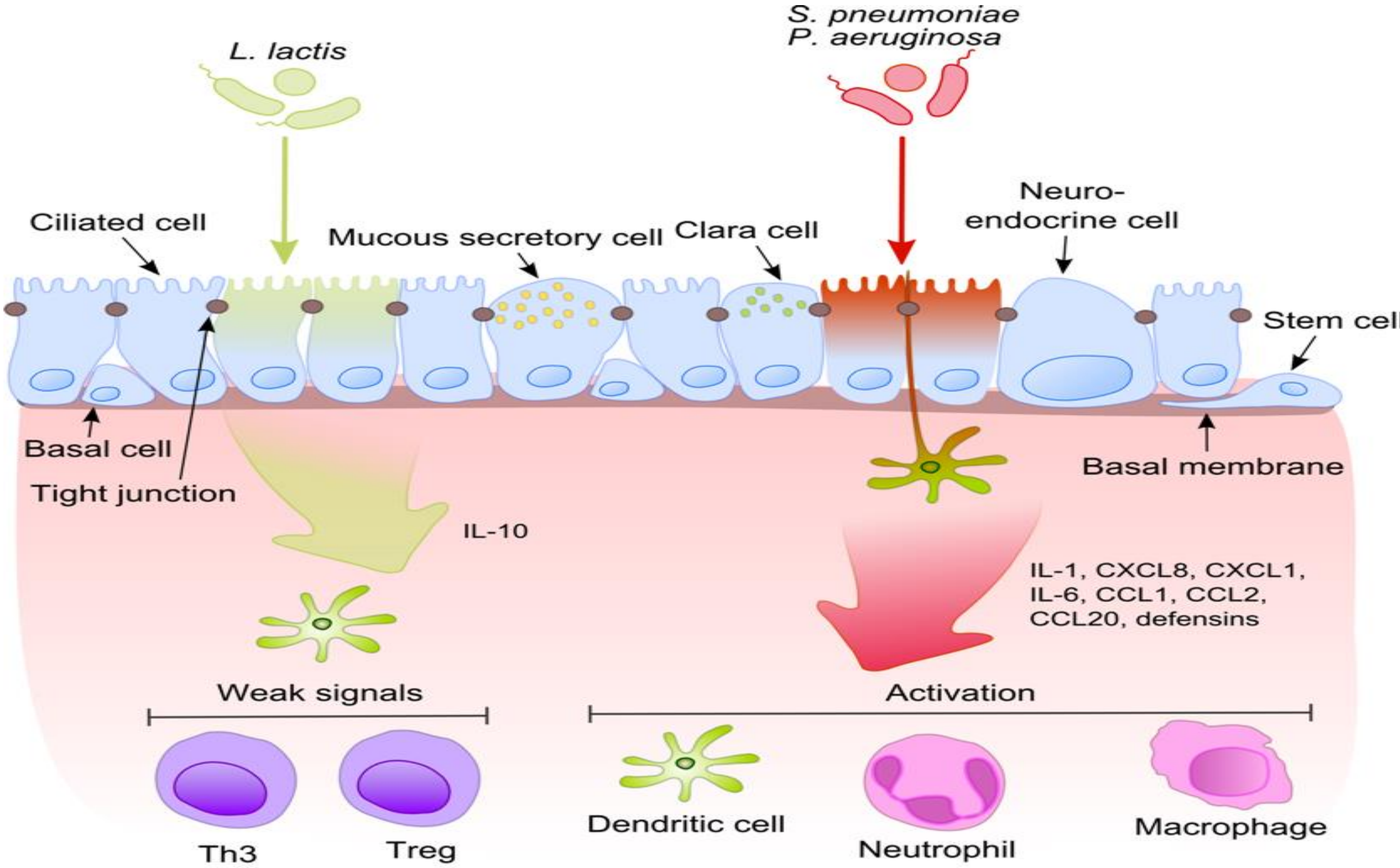


Nature Reviews | Immunology


Antigen
Keratinocyte
Activated Langerhans cell
Inflammation
Migration to local lymph node
Epidermis
Cytokine and chemokine release by LCs and keratinocytes
Activated dermal fibroblast
Innate effector-cell recruitment
Activated dermal dendritic cell
Migration to local lymph node
Dermis
E-selectin
ICAM1
Granulocyte
Chemokines
Monocyte/ macrophage/ NK cell
Naive T cell
Dermal post-capillary venule
Nature Reviews | Immunology

Skin

Normal Flora do not induce strong inflammatory responses



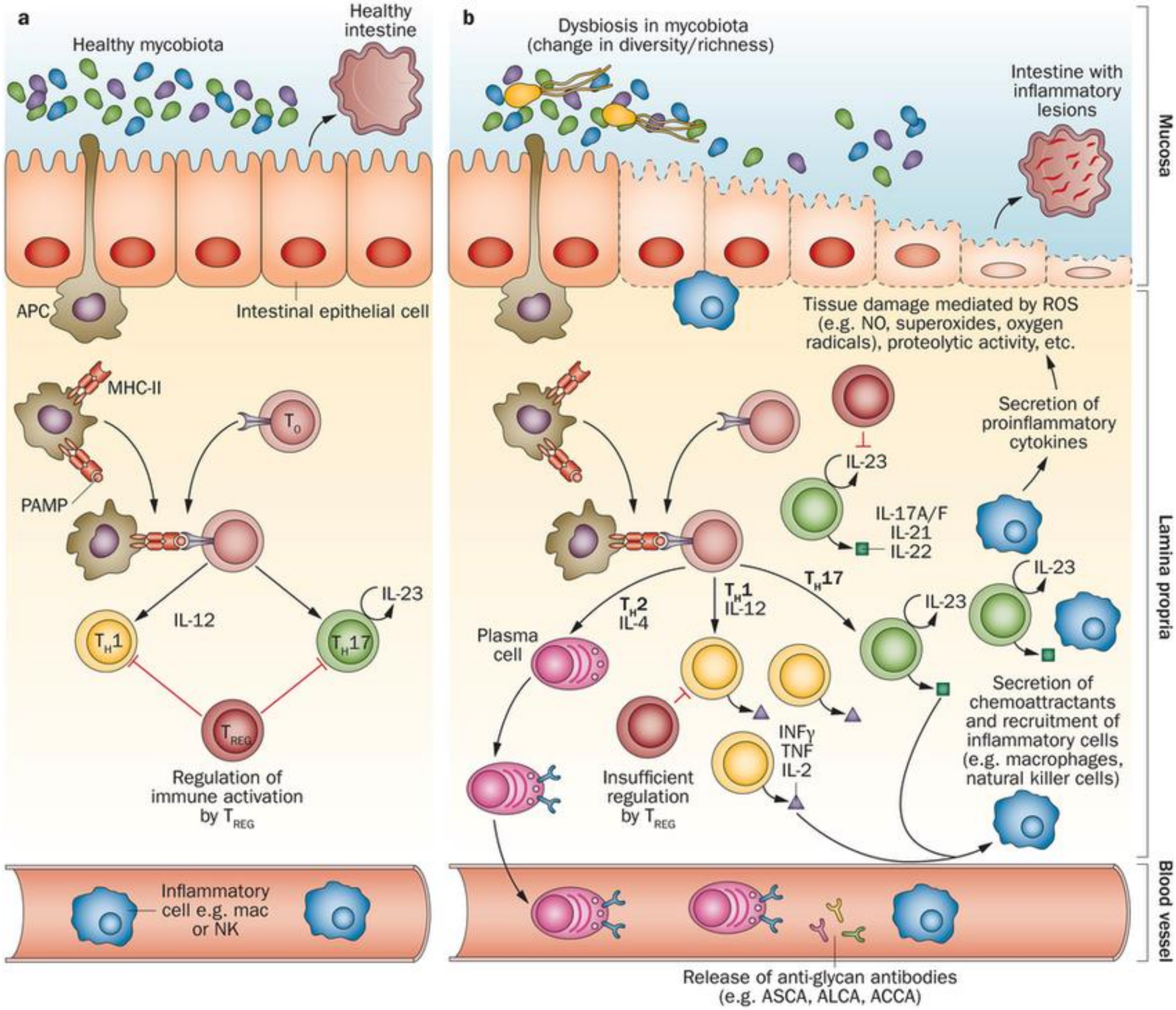
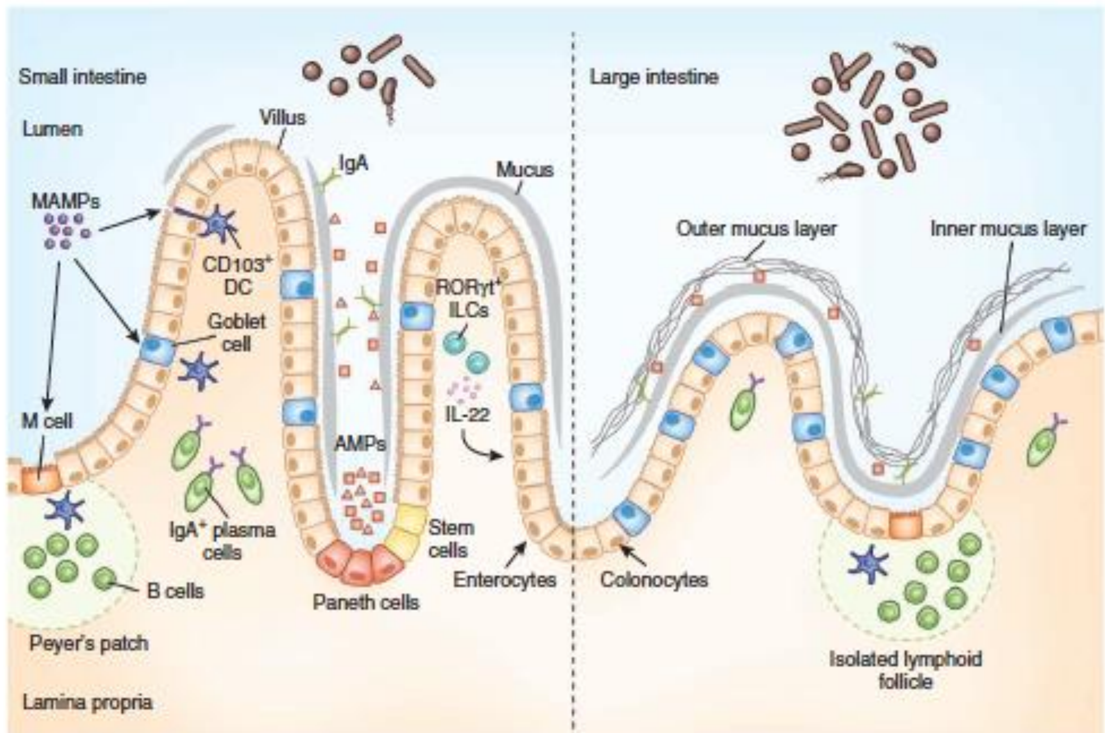

a
Healthy mycobiota
Healthy intestine
APC
Intestinal epithelial cell
MHC-II
PAMP
T_0
IL-12
T_H1
T_H17
IL-23
T_{REG}
Regulation of immune activation by T_{REG}
Inflammatory cell e.g. mac or NK
b
Dysbiosis in mycobiota (change in diversity/richness)
Intestine with inflammatory lesions
Tissue damage mediated by ROS (e.g. NO, superoxides, oxygen radicals), proteolytic activity, etc.
Secretion of proinflammatory cytokines
IL-23
IL-17A/F
IL-21
IL-22
T_H17
T_H1
IL-12
T_H2
IL-4
Plasma cell
IL-23
IL-23
INFγ
TNF
IL-2
Insufficient regulation by T_{REG}
Secretion of chemoattractants and recruitment of inflammatory cells (e.g. macrophages, natural killer cells)
Release of anti-glycan antibodies (e.g. ASCA, ALCA, ACCA)
Mucosa
Lamina propria
Blood vessel

Figure 2 Anatomical containment of the microbiota along the intestine. The intestinal epithelium comprises a single layer of enterocytes or colonocytes, and it is the role of the immune system to protect the integrity of this barrier. In the small intestine, absorptive requirements for enterocytes results in a discontinuous mucus layer, with fewer goblet cells. Here Paneth cells are enriched in the crypts, secreting AMPs, which can cross-link with the mucus layer. Through this barrier, sampling of MAMPs can be mediated through antigen uptake by M cells and goblet cells to dendritic cells (DCs), along with direct trans-epithelial luminal sampling from DCs. RORγt ILCs can sense microbial signals and produce IL-22 to aid in IEC barrier function. Commensal-specific IgA is produced by plasma cells in the lamina propria, mediated by DCs in a T cell–independent mechanism. The large intestine uses a thick, continuous mucus layer to compartmentalize the microbiota, with IgA and AMPs having a secondary role.



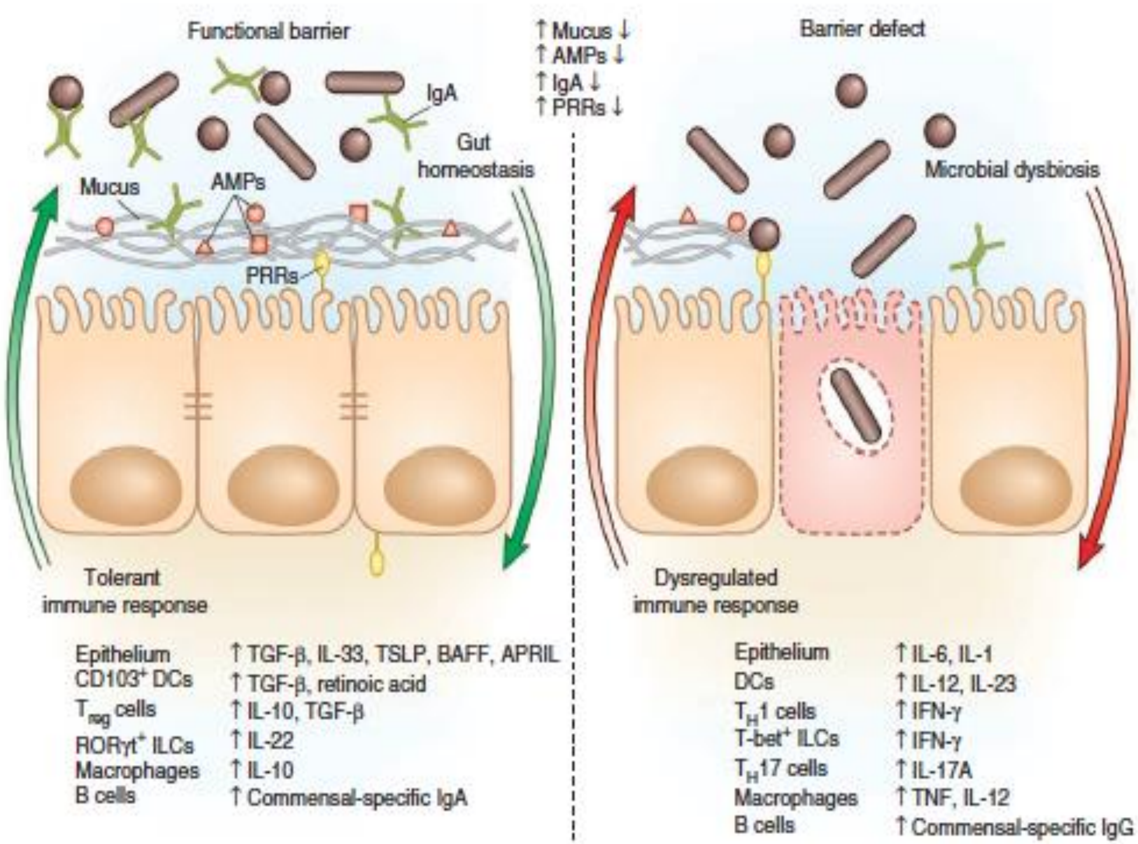
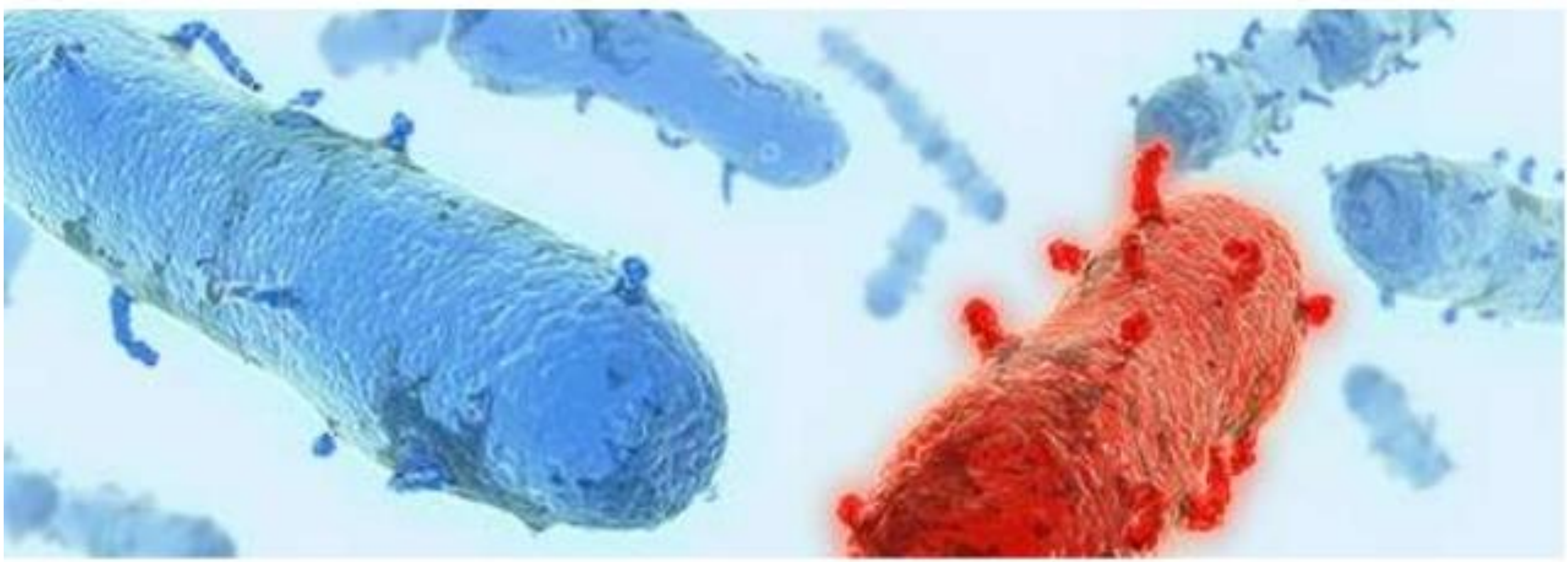
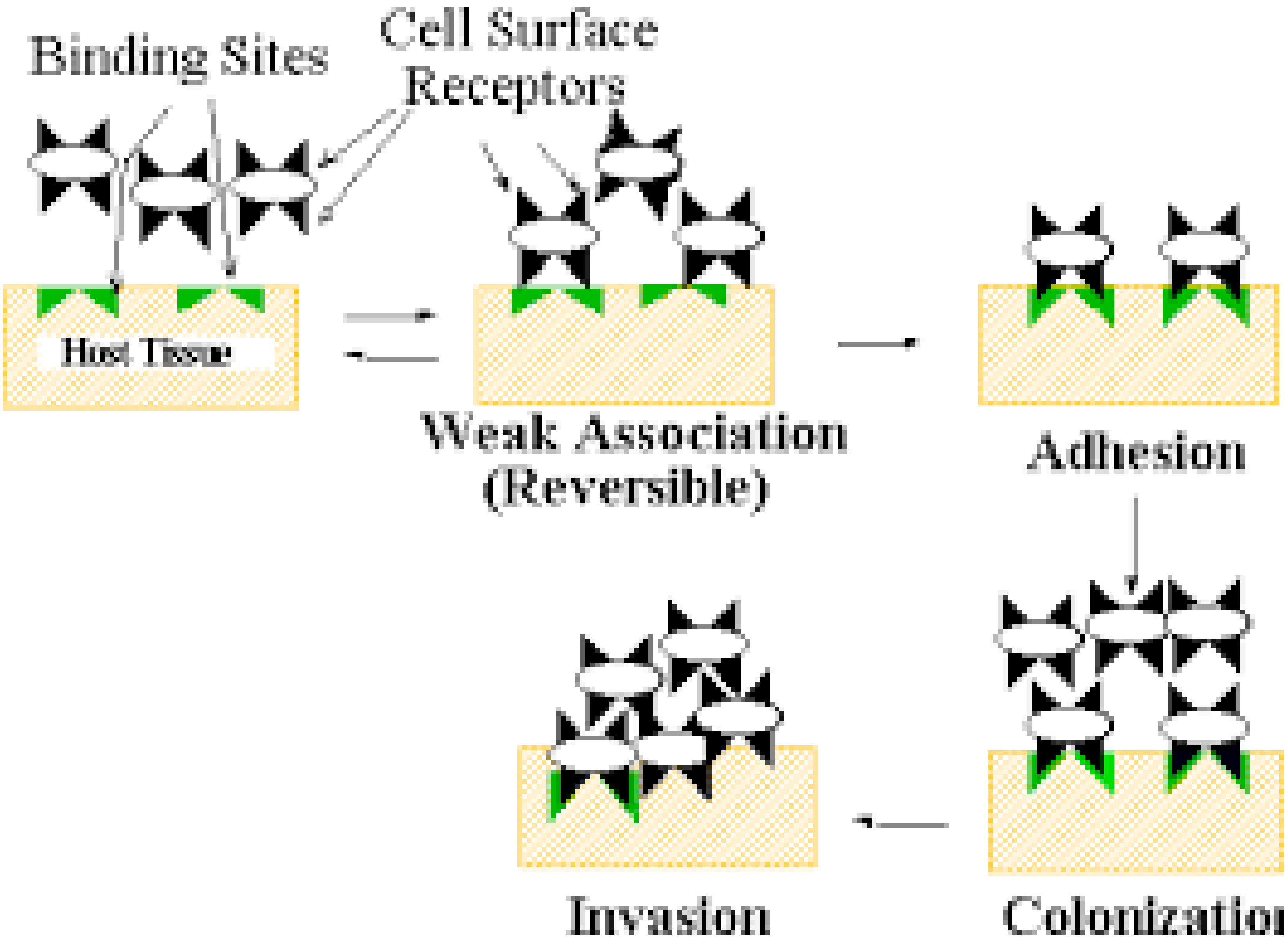


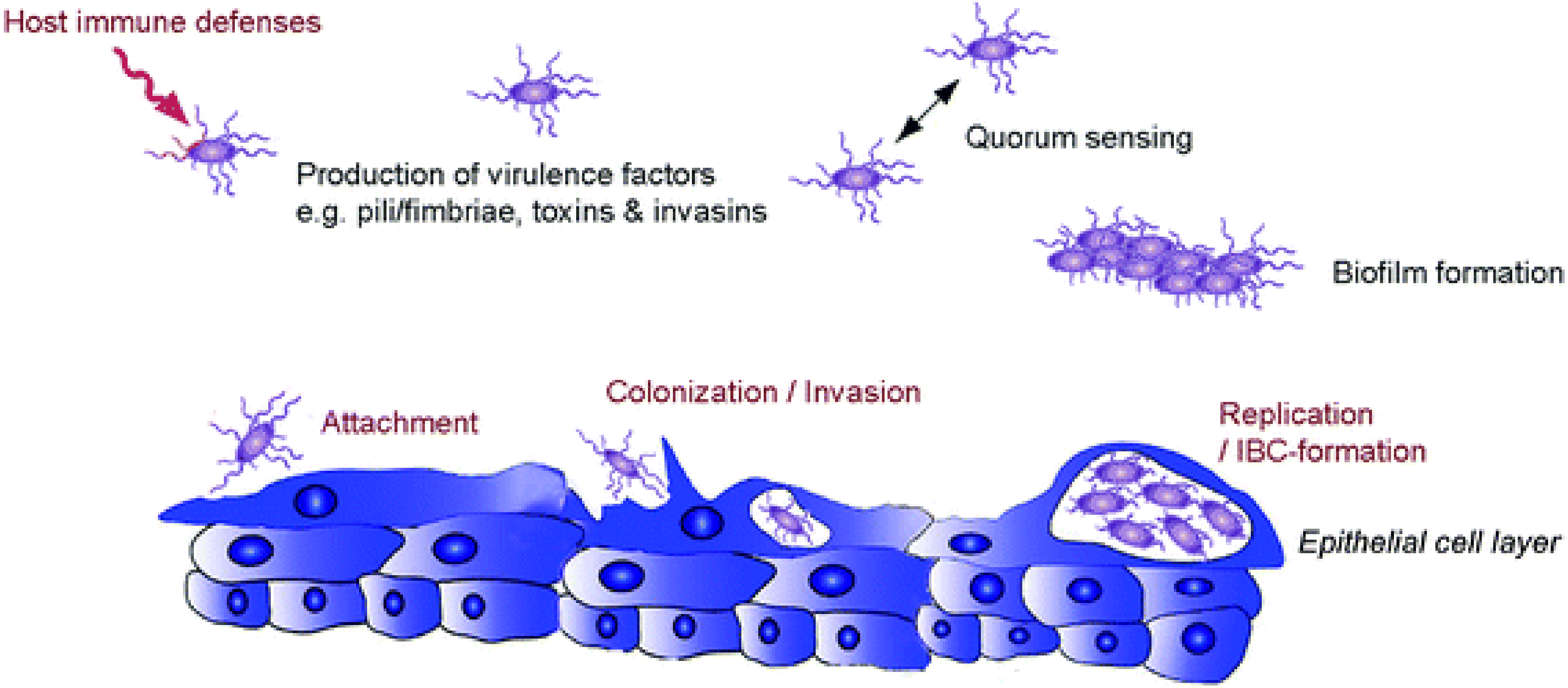
Figure 3 Innate barriers ensure a tolerant response to the microbiota. The presence of a functional barrier, with normal amounts of PRRs, mucus, AMPs and secreted IgA, promotes intestinal homeostasis with the microbiota. The microbiota is segregated away from the IECs, and the intestinal immune system directs a largely tolerant response to the resident commensals. MAMPs stimulate the epithelial secretion of IL-33, TGF-β, TSLP, BAFF and APRIL, all promoting the development of tolerogenic immune cell responses to the microbiota. This cytokine environment enriches for CD103+ dendritic cells (DCs), which aid in the development of T_{reg} cells secreting IL-10 and TGF-β. T_{reg} cells and CD103+ DCs stimulate the production of commensal-specific IgA. Barrier integrity of the IECs is enhanced by secretion of IL-22 by RORγt ILCs in this environment. In immunodeficiency or inflammatory syndromes with an innate barrier defect (for example, IBD, CVID or HIV infection), the intestinal immune system directs a potentially harmful pro-inflammatory response to the microbiota to clear invading bacteria and dysbiosis occurs. In this environment, the epithelium can secrete IL-1 and IL-6 in response to danger signals. Secretion of IL-12 and IL-23 by DCs and macrophages promote a T_H1 and T_H17 response. These T helper cells secrete high levels of IFN-γ and IL-17A, respectively, and T-bet+ ILCs also accumulate to produce IFN-γ. A breach in the epithelial barrier by the microbiota in this situation can also lead to higher levels of B cells secreting commensal-specific IgG.

Adhesion, invasion, colonization

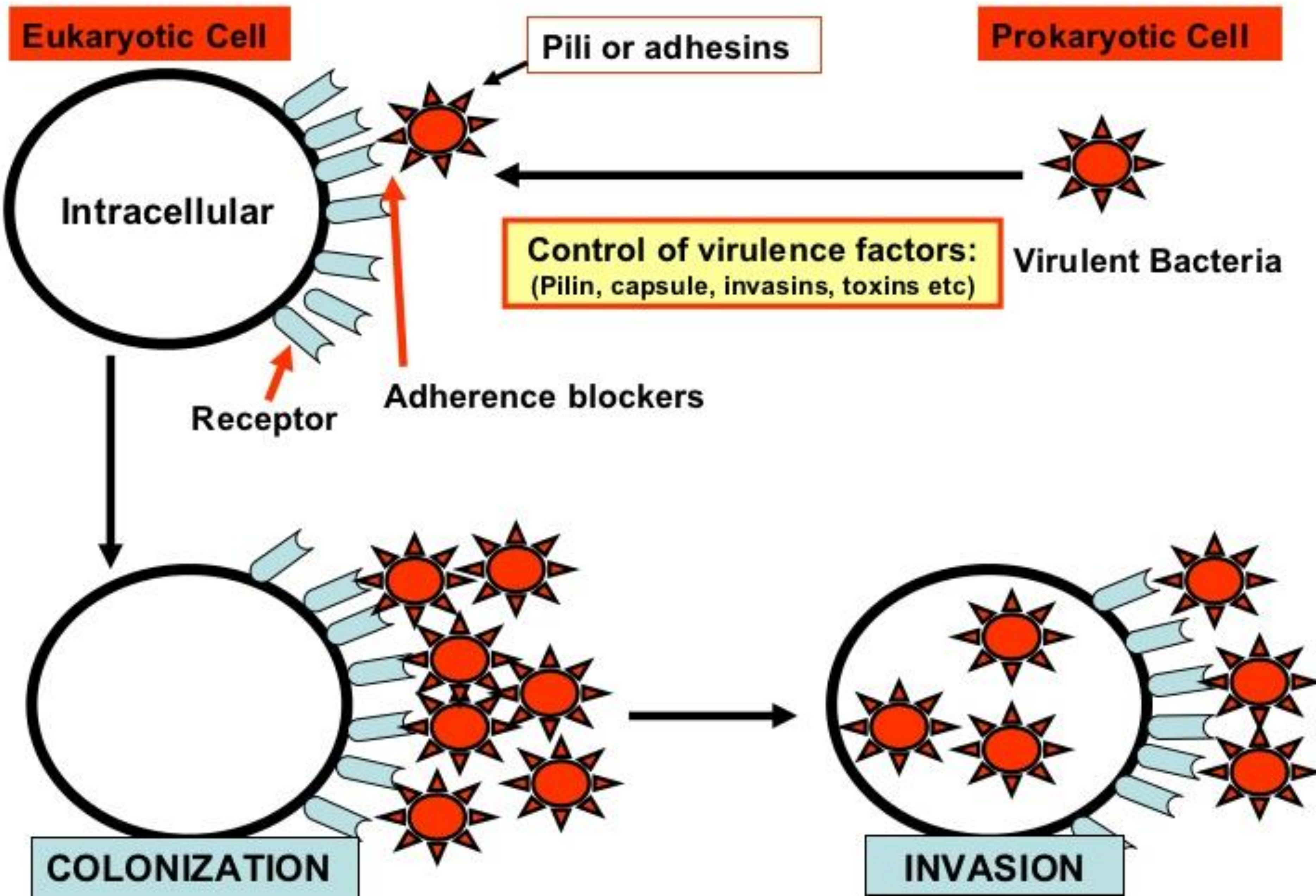


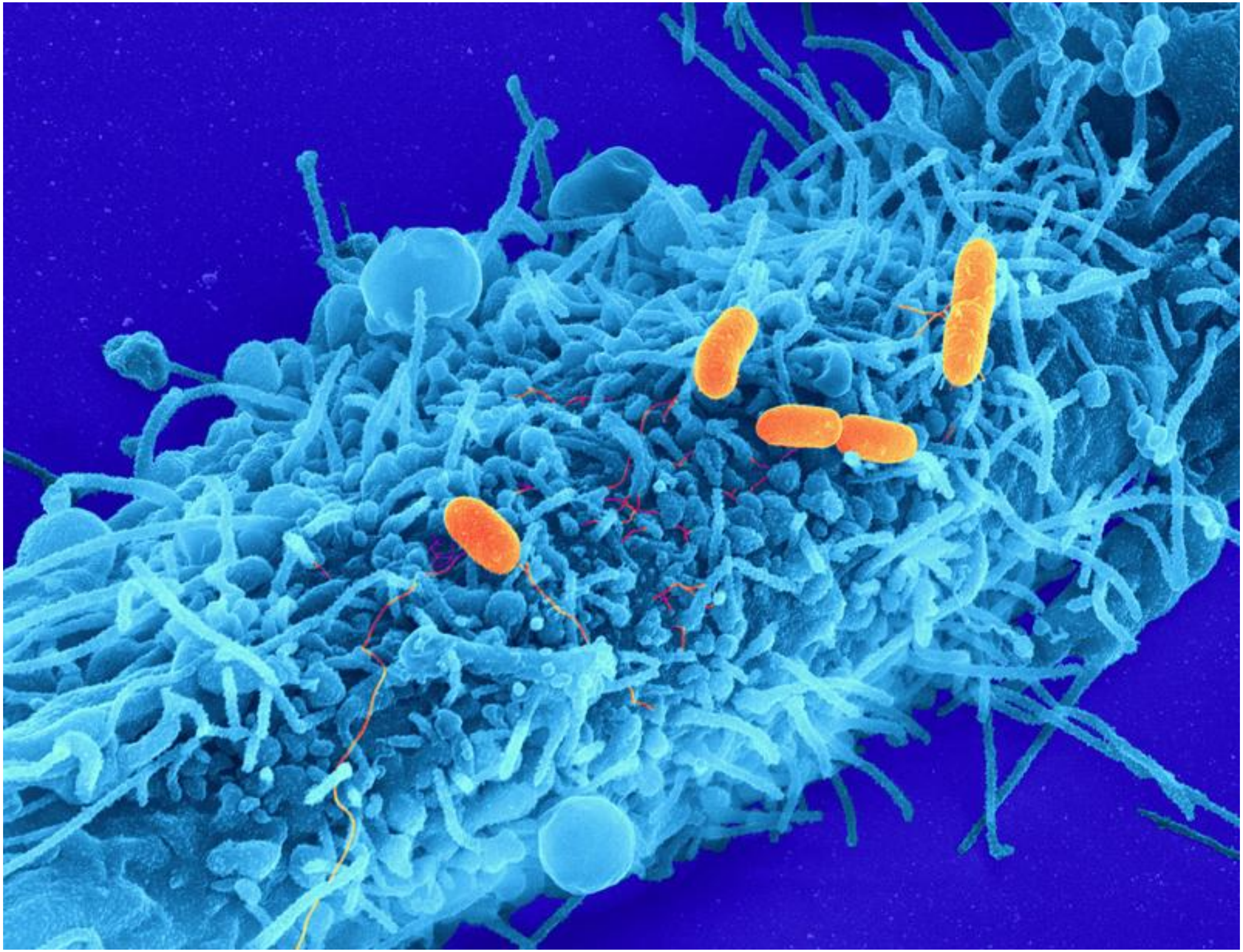


Binding Sites
Cell Surface
Receptors
Host Tissue
Weak Association
(Reversible)
Adhesion
Invasion
Colonizatio

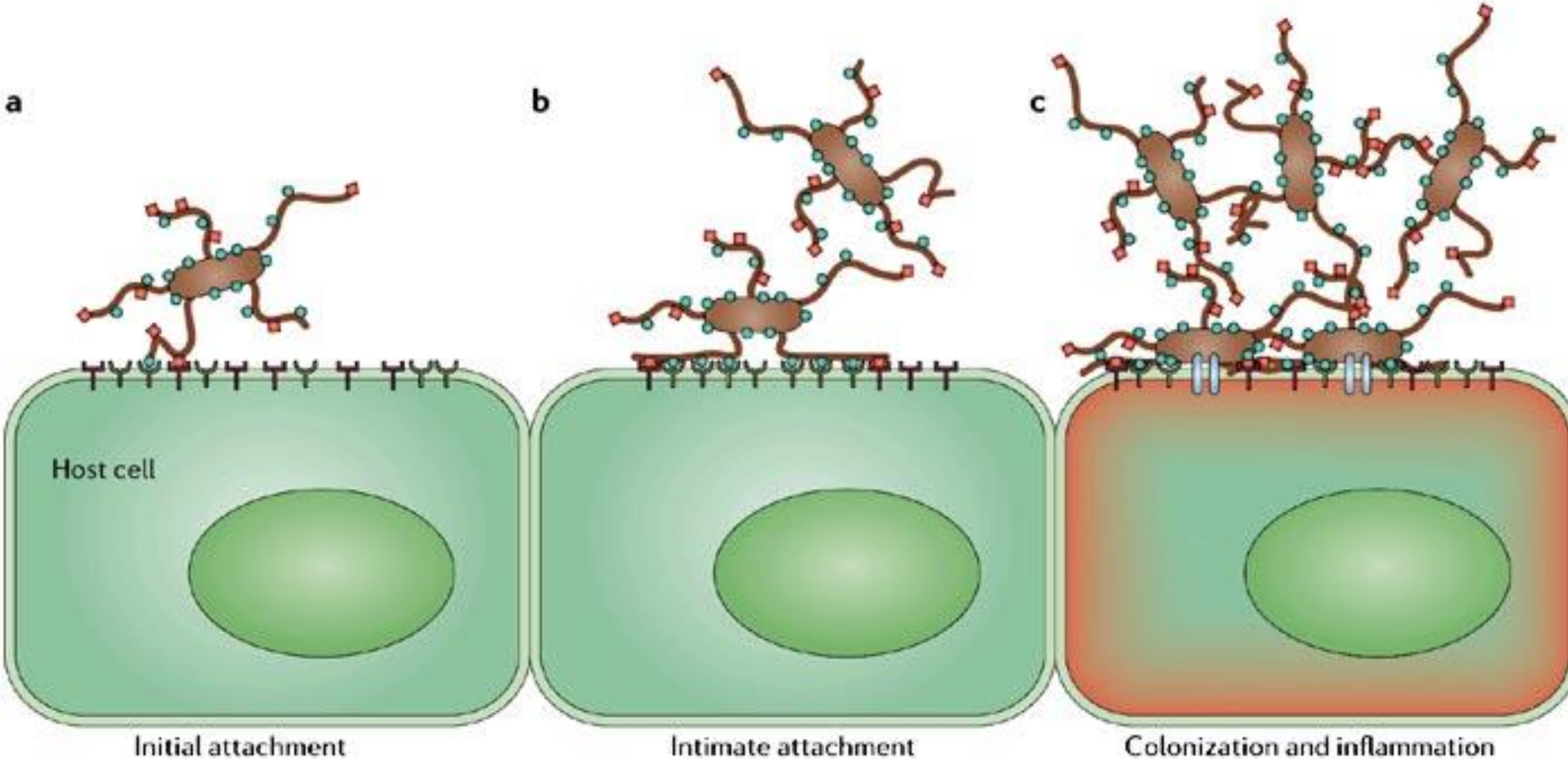

Host immune defenses
Production of virulence factors
e.g. pili/fimbriae, toxins & invasins
Quorum sensing
Biofilm formation
Attachment
Colonization / Invasion
Replication
/ IBC-formation
Epithelial cell layer

Prokaryotic and Eukaryotic Interactions

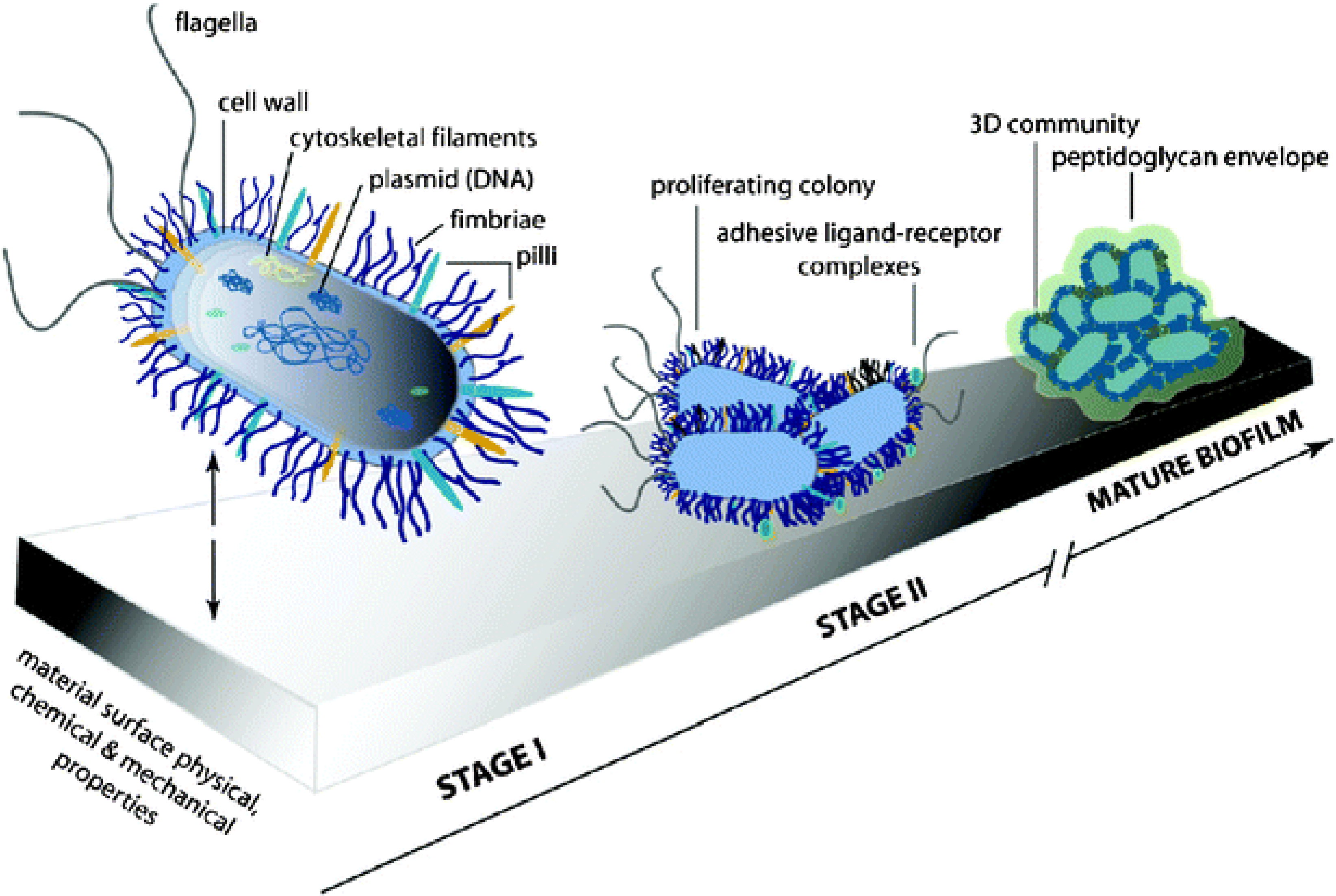


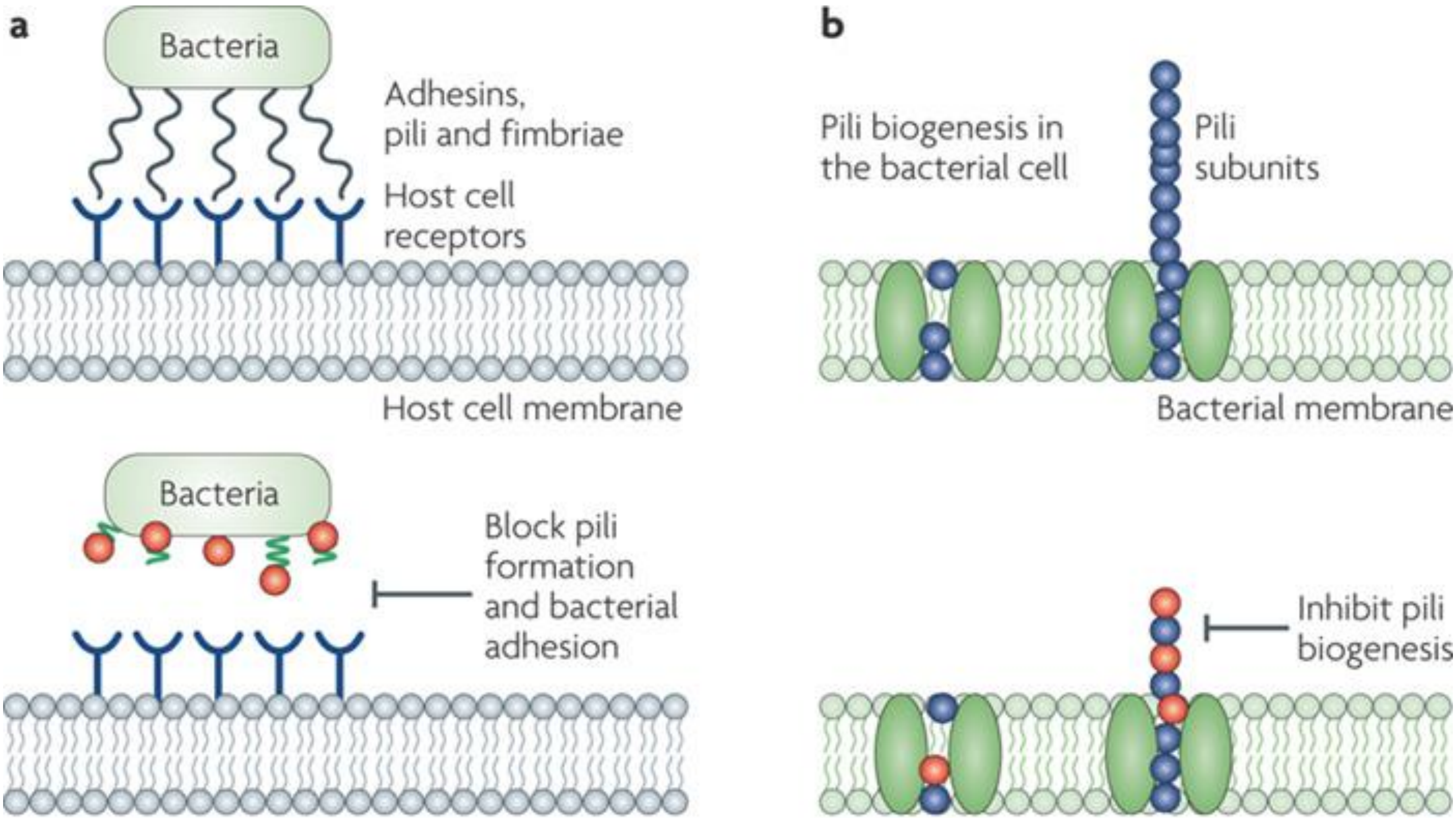


Bacterial infection of host cells: Pathogens of the type Salmonella typhimurium (orange) establish contact to a human host cell (blue).



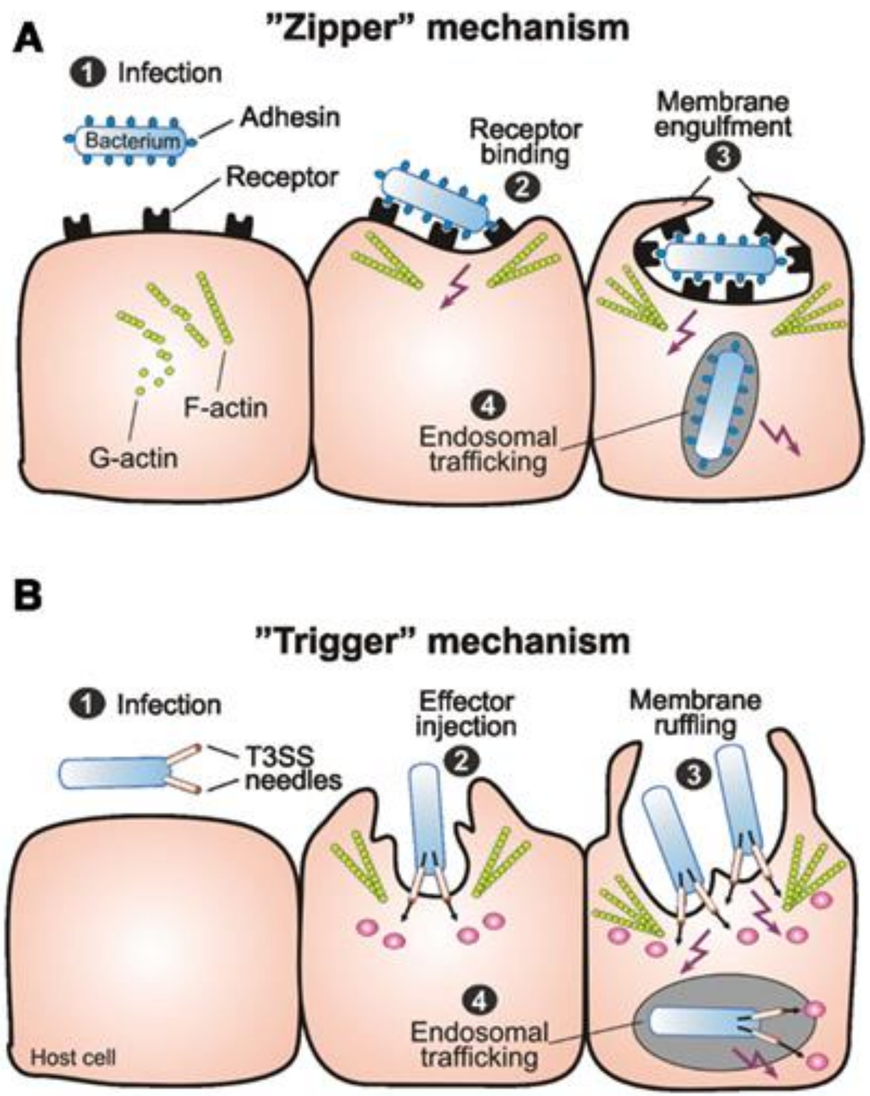
Nature Reviews | Microbiology


flagella
cell wall
cytoskeletal filaments
plasmid (DNA)
fimbriae
pilli
proliferating colony
adhesive ligand-receptor complexes
3D community
peptidoglycan envelope
MATURE BIOFILM
STAGE II
STAGE I
material surface physical, chemical & mechanical properties

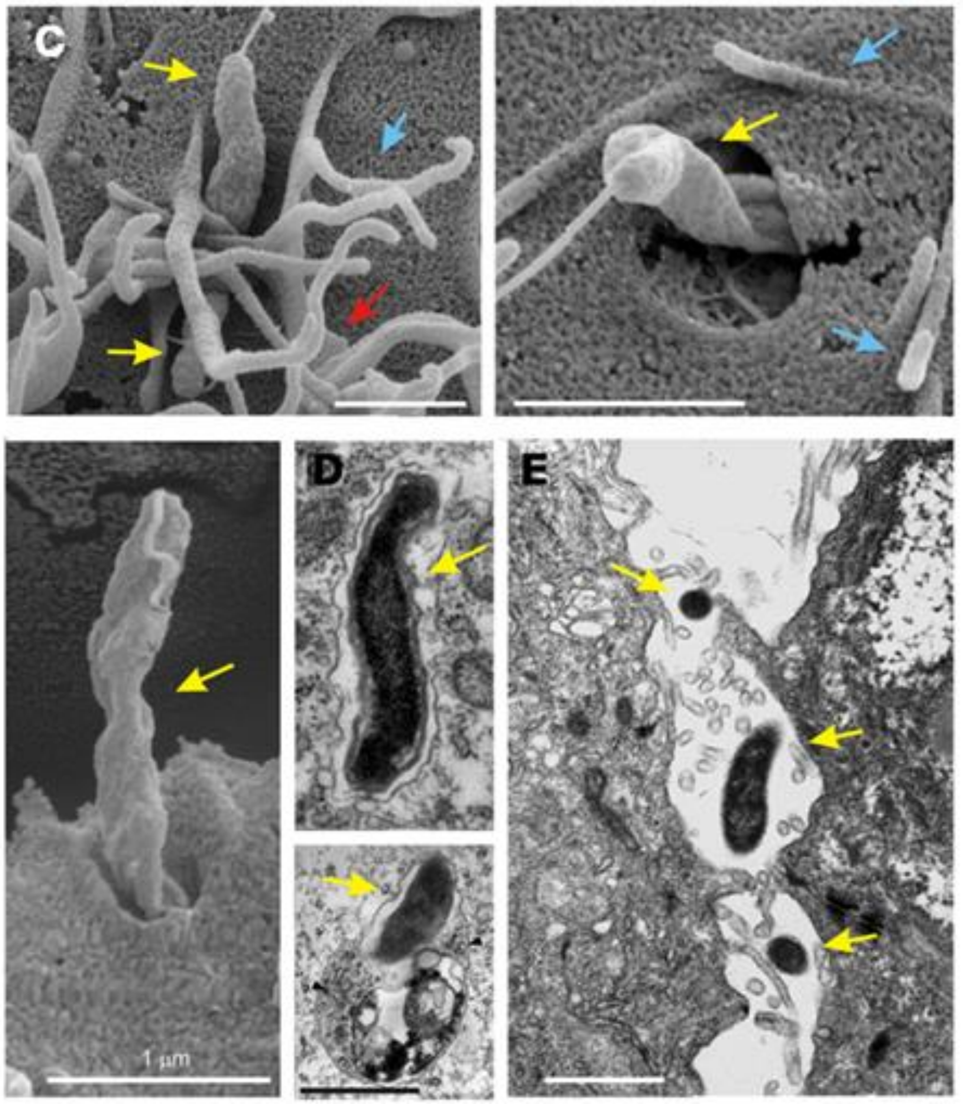


Nature Reviews | Drug Discovery

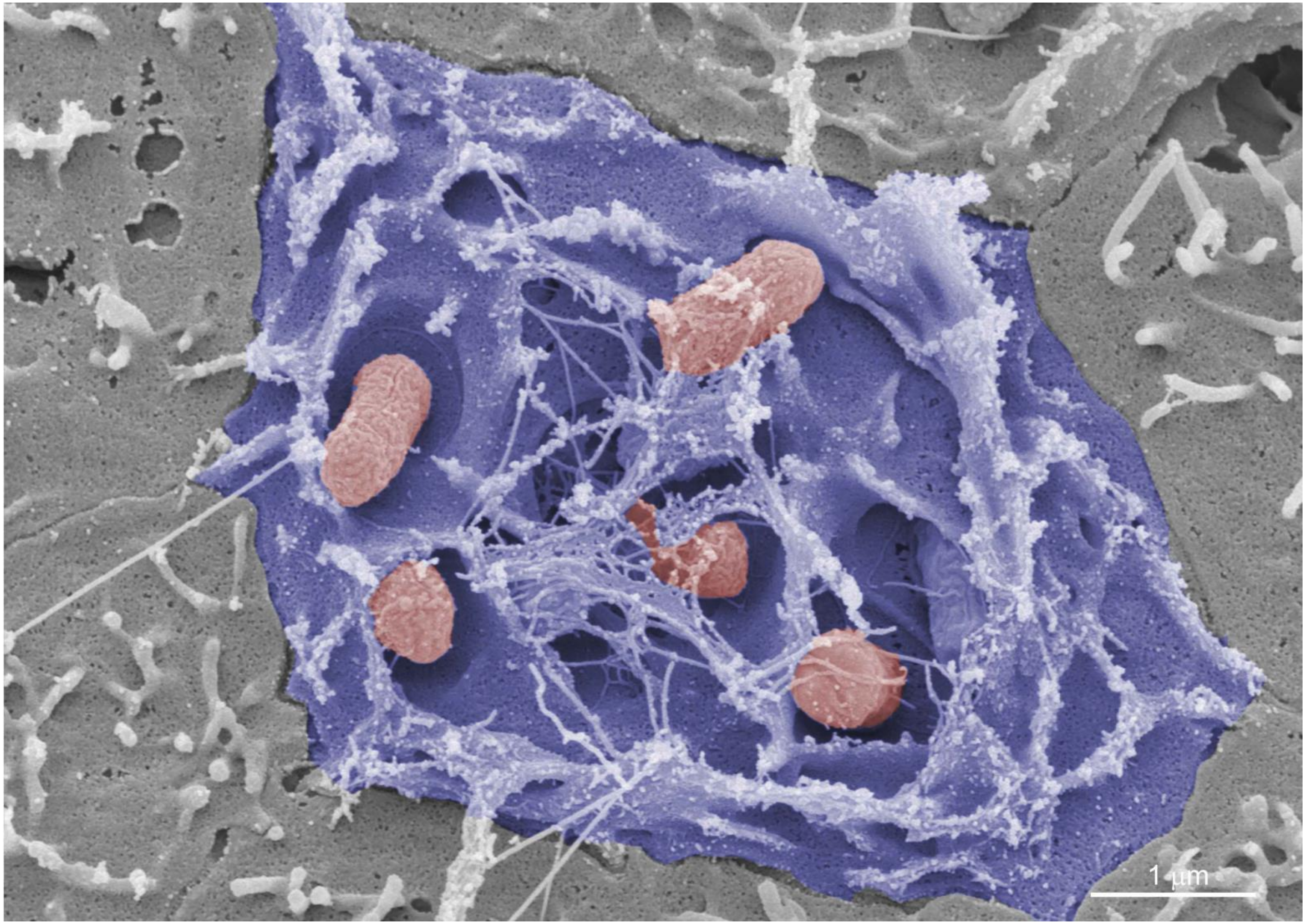
INVASION



A
"Zipper" mechanism
1 Infection
Adhesin
Bacterium
Receptor
Receptor binding
2
Membrane engulfment
3
G-actin
F-actin
4
Endosomal trafficking
B
"Trigger" mechanism
1 Infection
T3SS needles
Effector injection
2
Membrane ruffling
3
4
Endosomal trafficking
Host cell



C
D
E
1 μm


1 µm

4-Toxins &Enzymes

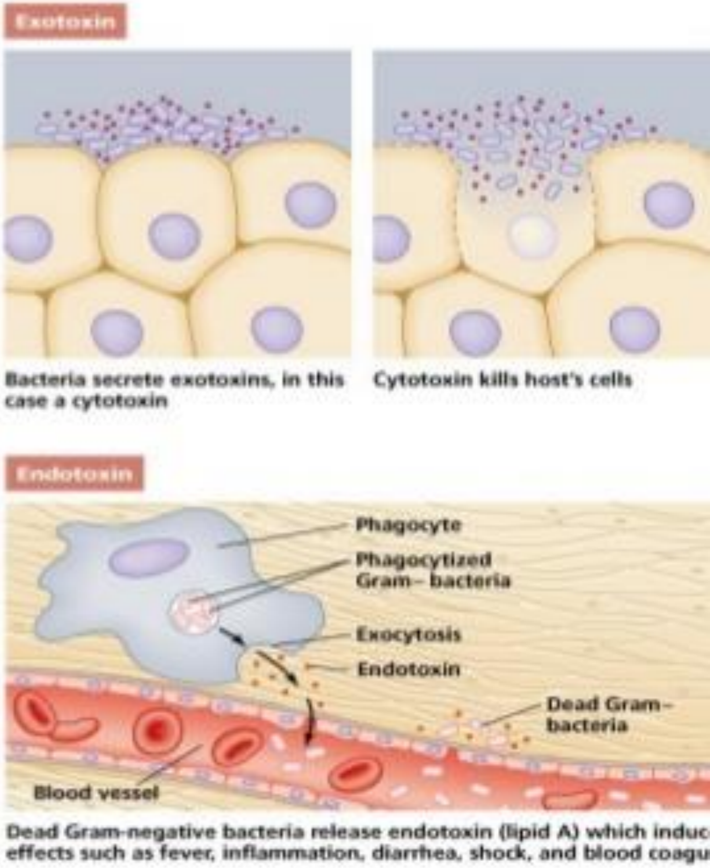
Toxins

- They are products of a pathogen that **destroy/ damage/**
- **inactivate** one or more vital component of the host.
- Classes of toxins

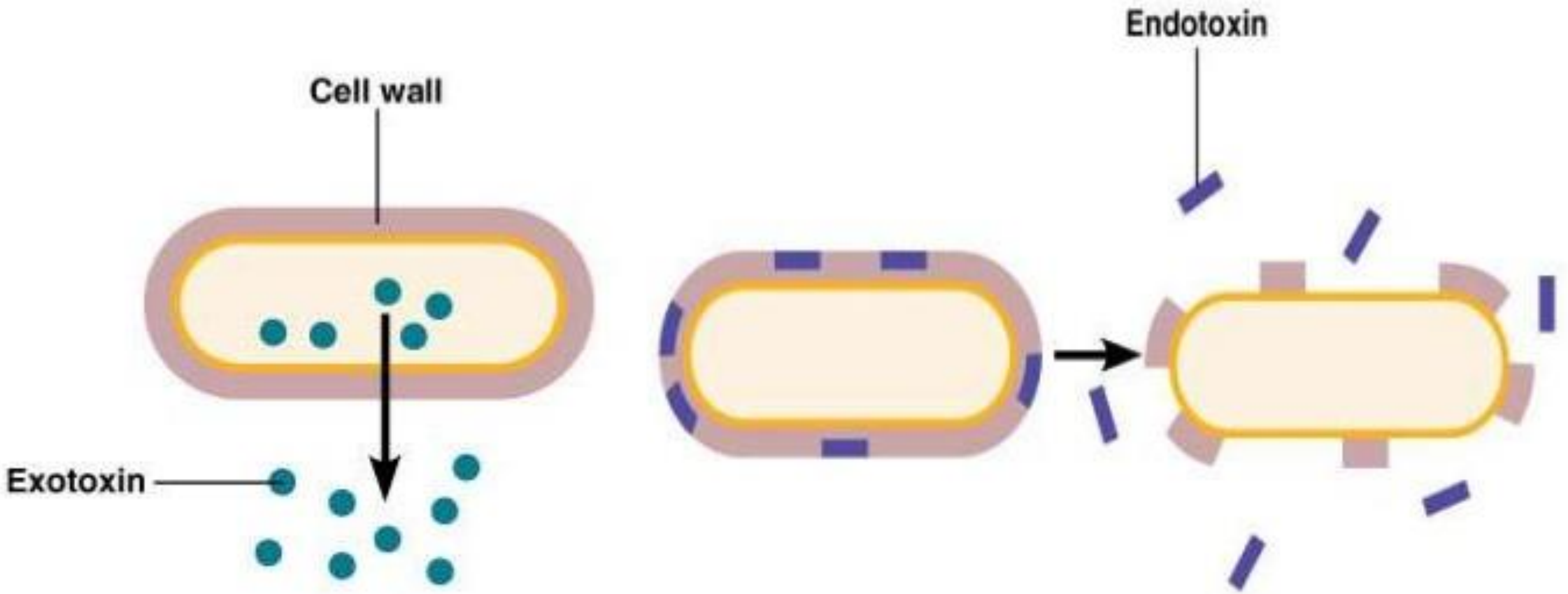
Neurotoxins

Enterotoxins

Cytotoxins



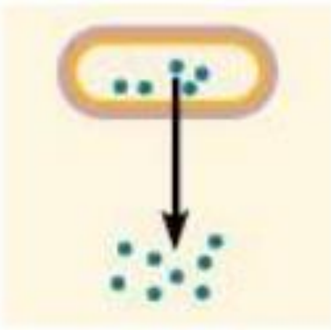
(b) Toxins



(a) Exotoxins are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then secreted or released following lysis into the surrounding medium.

(b) Endotoxins are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

Exotoxin

	
Source	Mostly Gram +
Metabolic product	By-products of growing cell
Chemistry	Protein
Fever?	No
Neutralized by antitoxin	Yes
LD_{50}	Small

Exotoxins

- Superantigens or type I toxins
  - Cause an intense immune response due to release of cytokines from host cells
  - Fever, nausea, vomiting, diarrhea, shock, death

- Membrane-disrupting toxins or type II toxins
  - Lyse host's cells by:
    - Making protein channels in the plasma membrane (e.g., leukocidins, hemolysins)
    - Disrupting phospholipid bilayer

Exotoxins

- A-B toxins or type III toxins

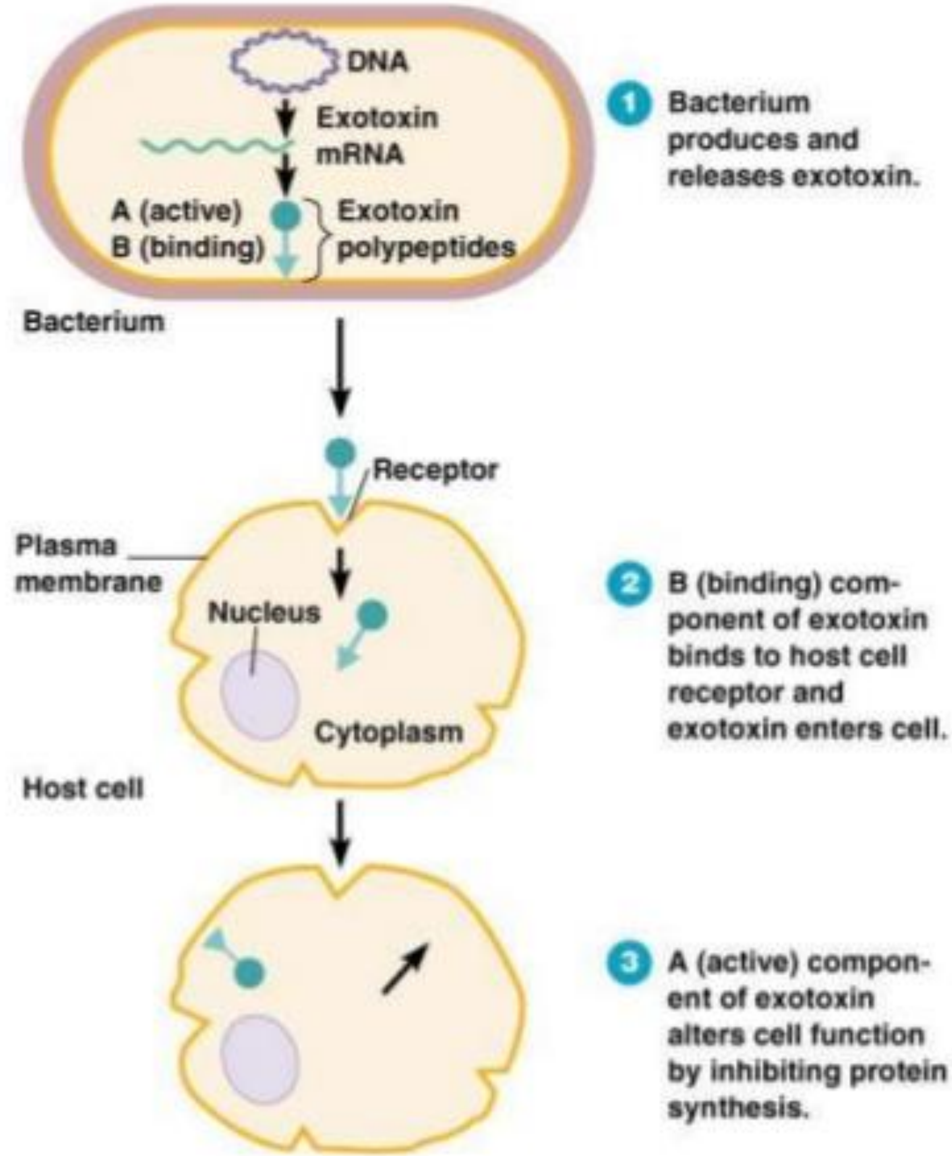
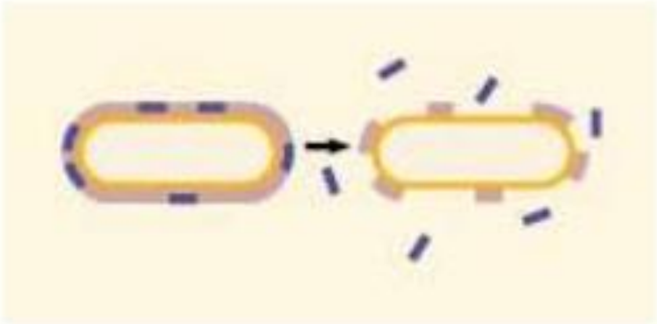


Figure 15.5

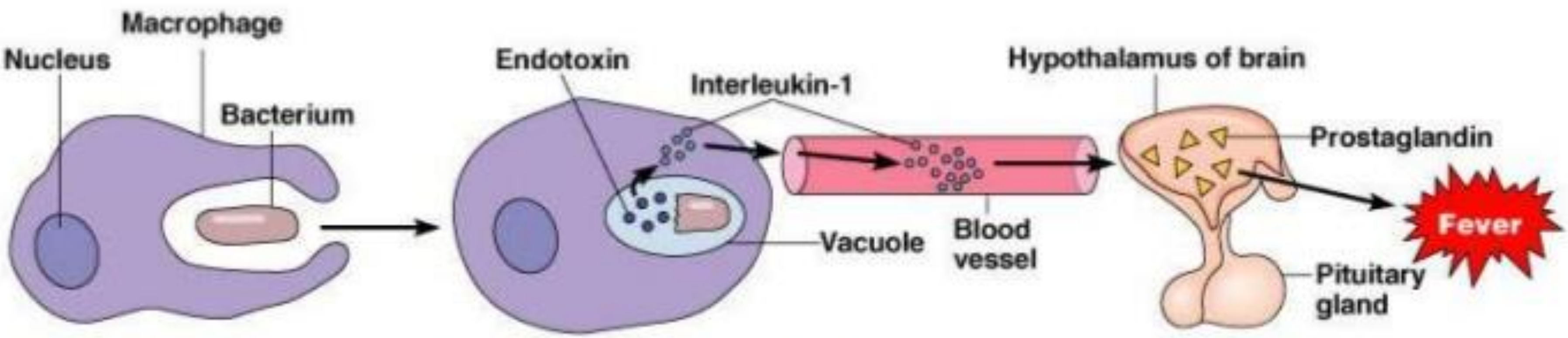
Exotoxins

	Exotoxin	Lysogenic conversion
• *Corynebacterium diphtheriae*	A-B toxin. Inhibits protein synthesis.	+
• *Streptococcus pyogenes*	Membrane-disrupting. Erythrogenic.	+
• *Clostridium botulinum*	A-B toxin. Neurotoxin	+
• *C. tetani*	A-B toxin. Neurotoxin	
• *Vibrio cholerae*	A-B toxin. Enterotoxin	+
• *Staphylococcus aureus*	Superantigen. Enterotoxin.	

Endotoxins

	
Source	Gram–
Metabolic product	Present in LPS of outer membrane
Chemistry	Lipid
Fever?	Yes
Neutralized by antitoxin	No
LD_{50}	Relatively large

Endotoxins



1. A macrophage ingests a gram-negative bacterium
2. The bacterium is degraded in a vacuole, releasing endotoxins that induce the macrophage to produce interleukin-1(IL-1)
3. IL-1 is released by the macrophage into the bloodstream, through which it travels to the hypothalamus of the brain
4. IL-1 induces the hypothalamus to produce prostaglandins, which reset the body's "thermostat" to a higher temperature, producing fever

Figure 15.6

Organism	Pilli	Function
Pnumococcus	1 RrgA pillus-associated adhesin 2 PspC	Adhesion Binds secretory component on the polymeric immunoglobulin receptor
Bordetella bronchiseptica	FHA	Adhesion
S. aureus	1 teichoic acids 2 Clumping factor B	binds the nasal cavity surface receptor SREC-I Binds loricrin, a protein found on the cornified envelope of the squamous epithelium in the anterior nares
Escherichia coli	pyelonephritis-associated (P) pili	PapG, that binds to a-D-galactopyranosyl- (1-4)-b-D-galactopyranoside glycosphingolipids of the kidney epithelium

Some Extra Cellular Bacterial Proteins That Act As Invasins:

Invasin	Bacteria Involved	Activity
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue
Collagenase	*Clostridium*species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae*and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	Staphylococcus aureus	Converts fibrinogen to fibrin which causes clotting

Kinases	**Staphylococci and streptococci**	**Converts plasminogen to plasmin which digests fibrin**
Leukocidin	*Staphylococcus aureus*	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	*Streptococcus pyogenes*	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules
Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis
Lecithinases	*Clostridium perfringens*	Destroy lecithin in cell membranes

Πολλαπλασιαμός βλαστικών/πολυδύναμων κυττάρων και αναγέννηση επιθυλίων σε περίπτωση μικροβιακής λοίμωξης και βλάβης του επιθυλίου

Regeneration

Regeneration refers to the proliferation of cells and tissues to replace lost structures

- Whole organs and complex tissues rarely regenerate after injury
  - Exceptions are liver, epithelia of GIT, Skin, Hemopoietic tissue
- Compensatory growth Vs Regeneration

Repair

- Repair is a healing process
- *It's a combination of regeneration* and *scar formation*

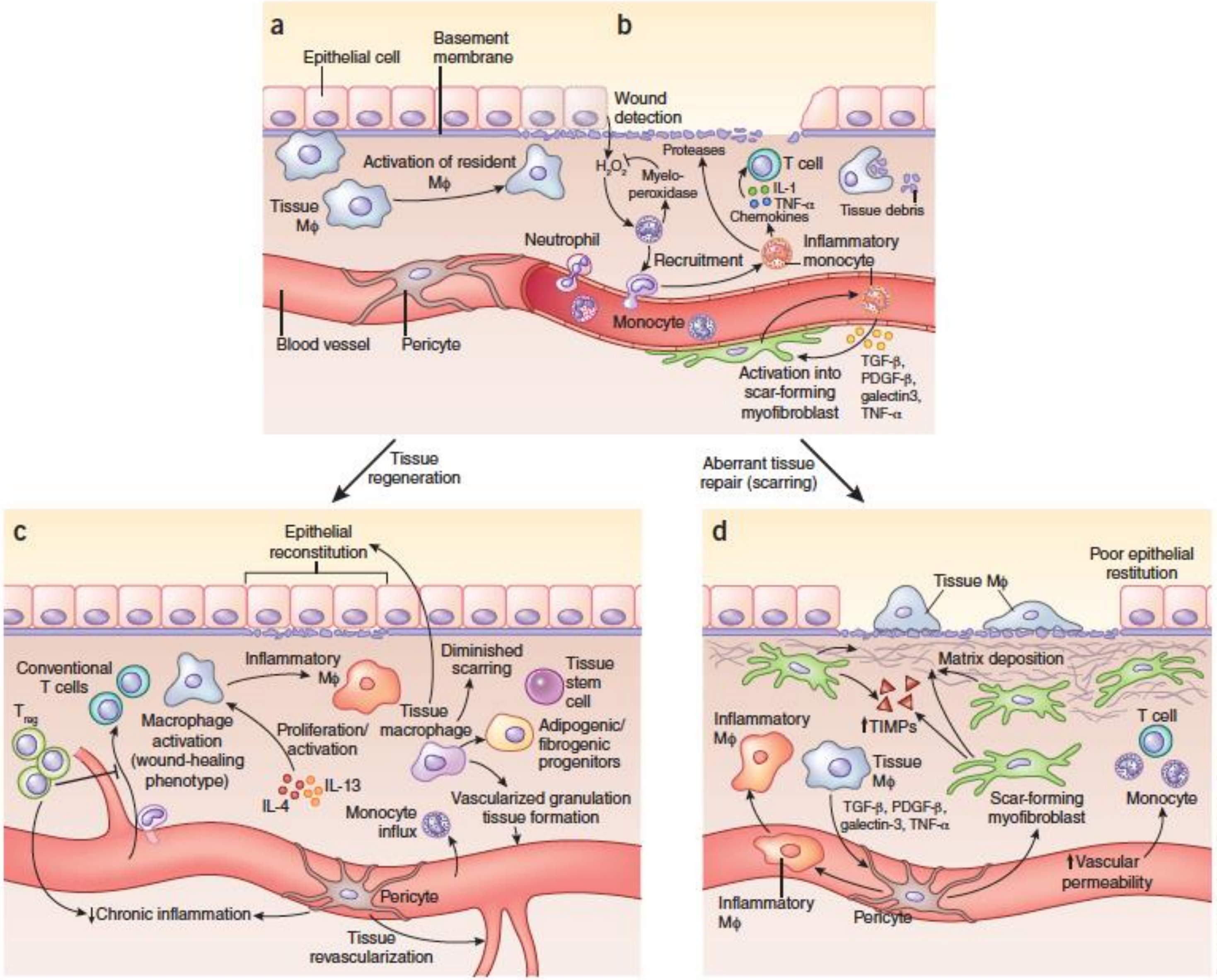
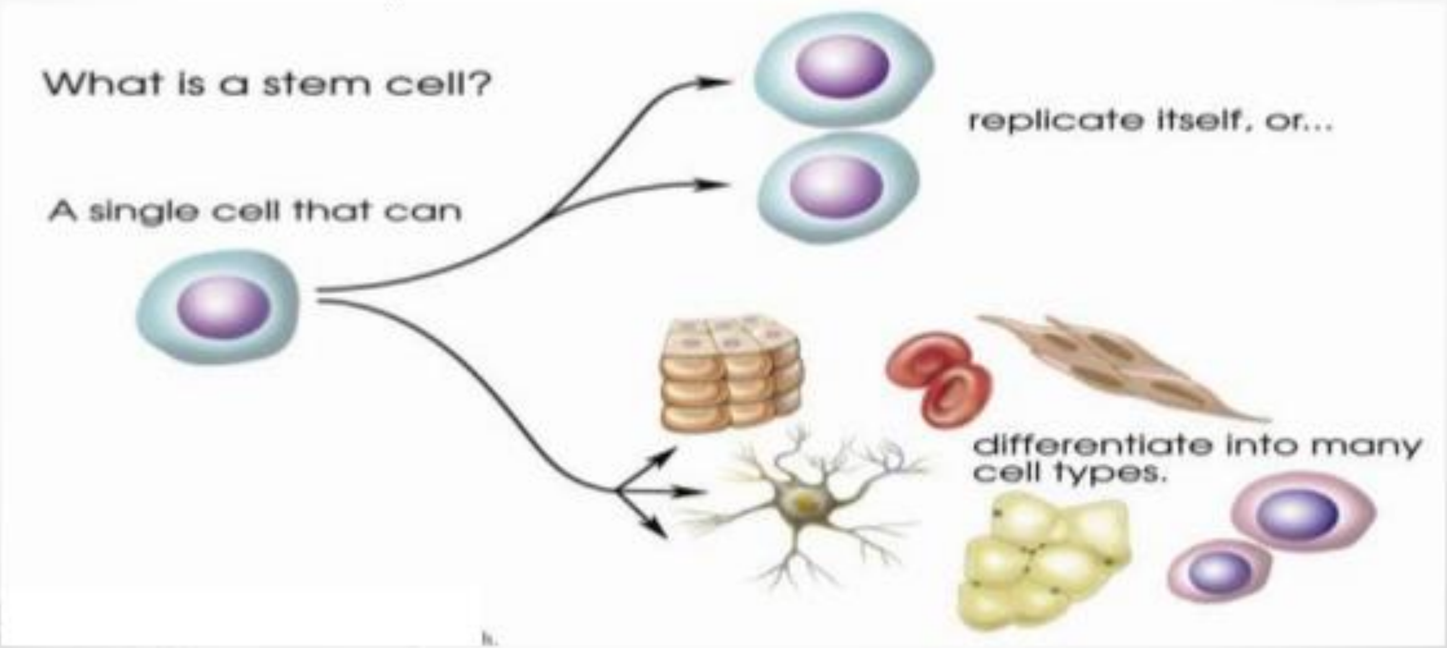


Figure 1 General model of tissue repair. (**a**) In healthy tissue, there is little monocyte trafficking or neutrophil efflux into tissues, an intact basement membrane and no scar tissue. (**b**) Upon tissue damage, there is loss of epithelia, neutrophil influx, activation of resident macrophages (Mϕ) and recruitment of inflammatory monocytes, in addition to release of inflammatory factors and activation of pericytes into myofibroblasts. (**c**) During tissue regeneration, there is epithelial proliferation for reconstitution, macrophage activation into a wound-healing phenotype and matrix remodeling. In addition, T_{reg} cells are recruited to decrease chronic inflammation. (**d**) During aberrant tissue repair, excessive scarring occurs as a result of continued activation of inflammatory cells and impaired epithelial regeneration. TIMPs, tissue inhibitor of metalloproteinases; PDGF-β, platelet-derived growth factor-β.

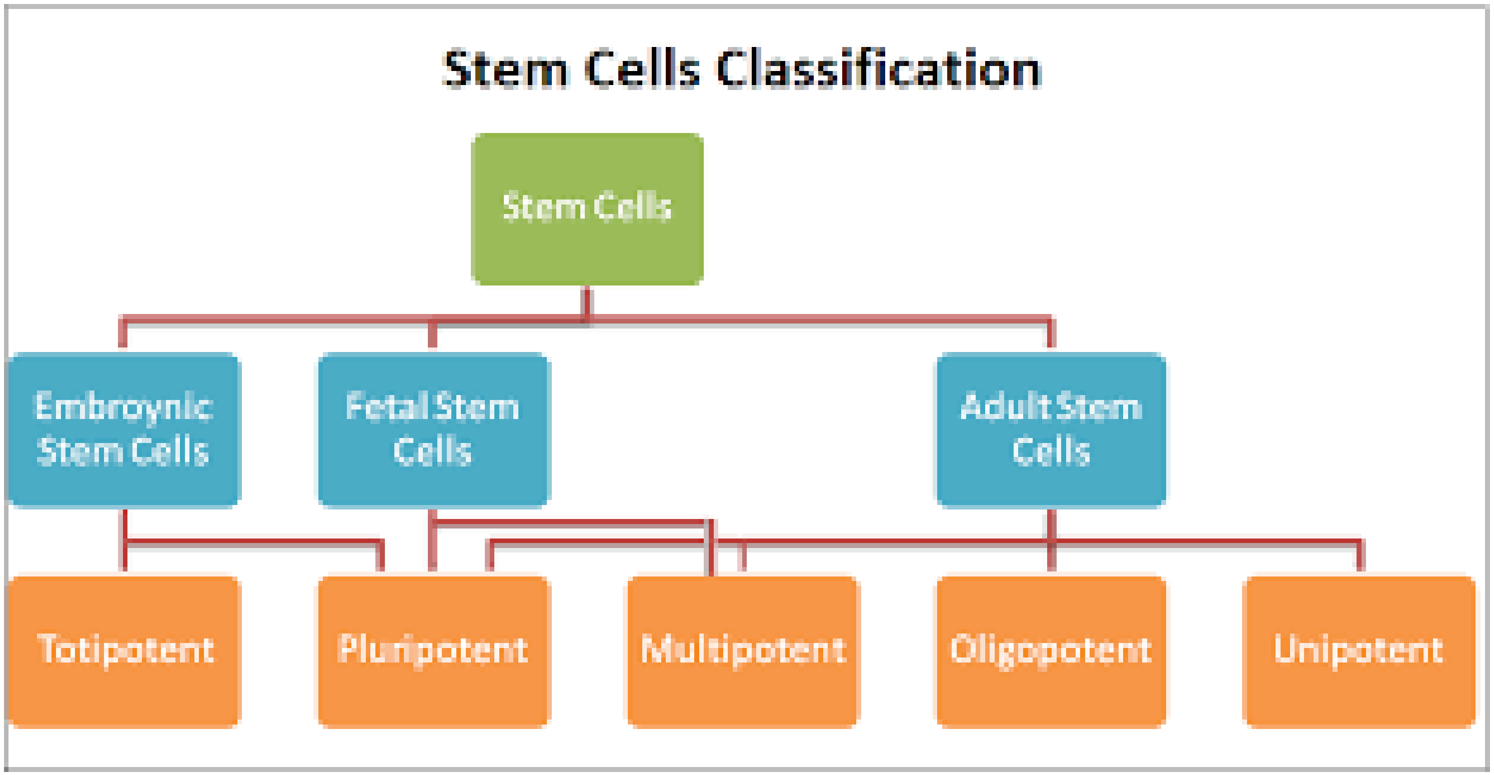
Stem cell

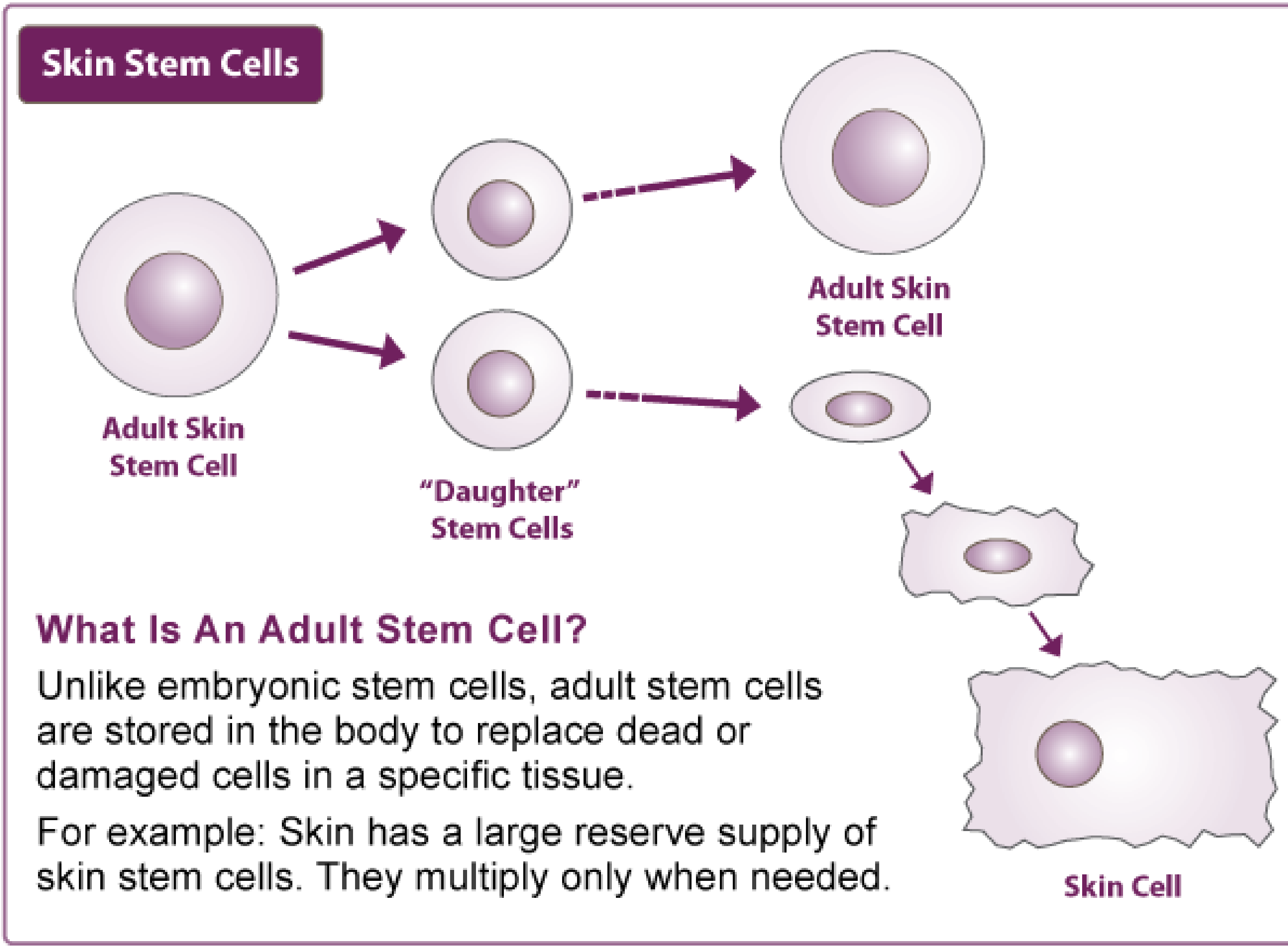
Definition

- A cell that has the ability to continuously divide and differentiate (develop) into various other kinds of cells/tissues.

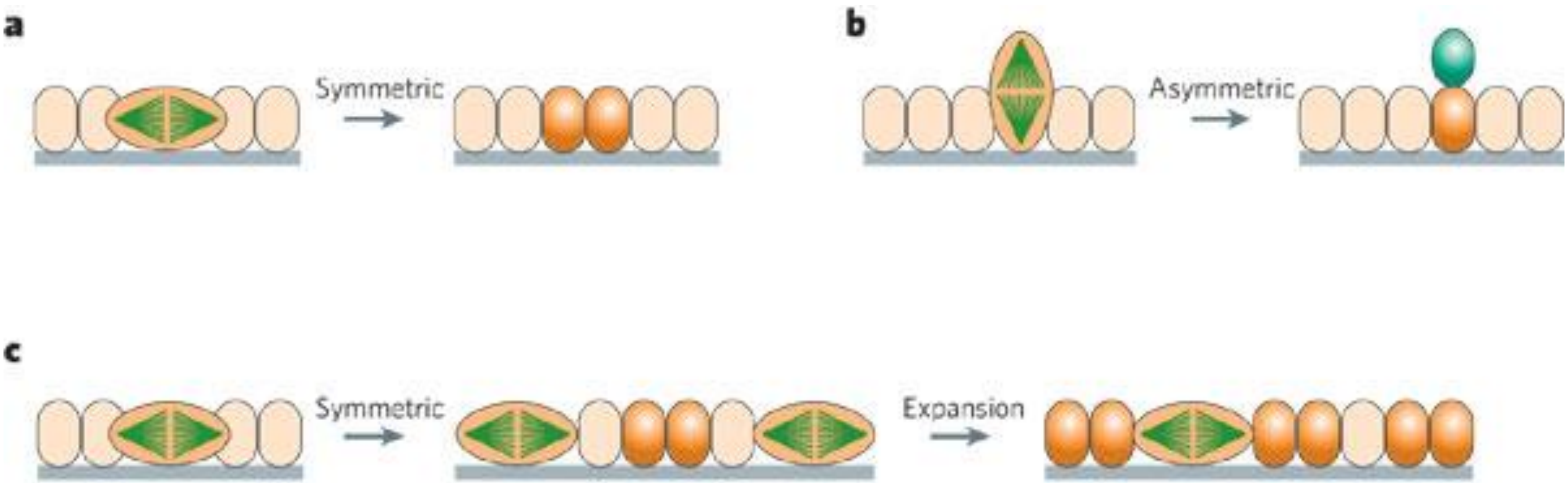



WHAT TYPES OF STEM CELLS ARE THERE?

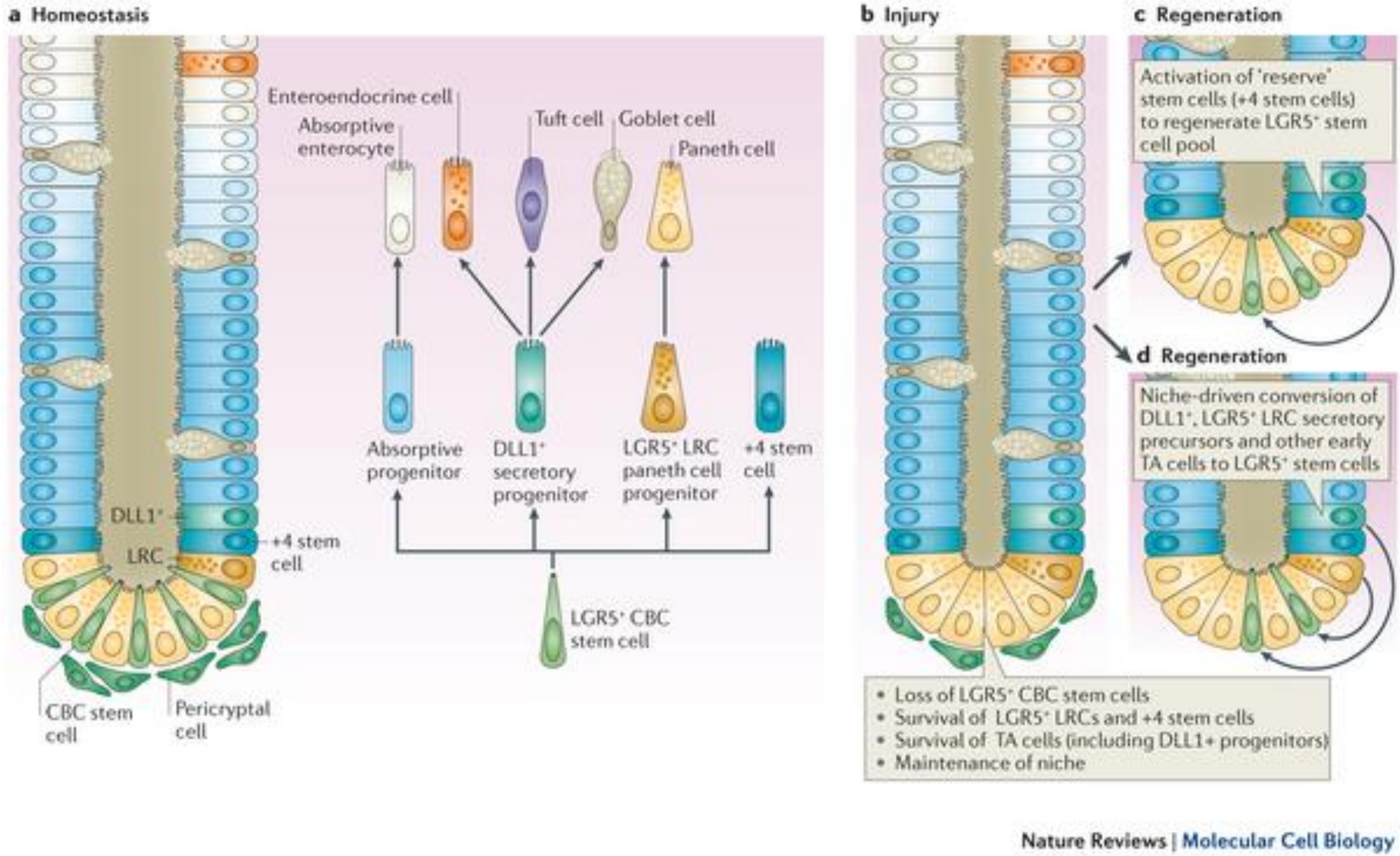

Stem Cells Classification
Stem Cells
Embroynic Stem Cells
Fetal Stem Cells
Adult Stem Cells
Totipotent
Pluripotent
Multipotent
Oligopotent
Unipotent


Skin Stem Cells
Adult Skin Stem Cell
"Daughter" Stem Cells
Adult Skin Stem Cell
Skin Cell
What Is An Adult Stem Cell?
Unlike embryonic stem cells, adult stem cells are stored in the body to replace dead or damaged cells in a specific tissue.
For example: Skin has a large reserve supply of skin stem cells. They multiply only when needed.

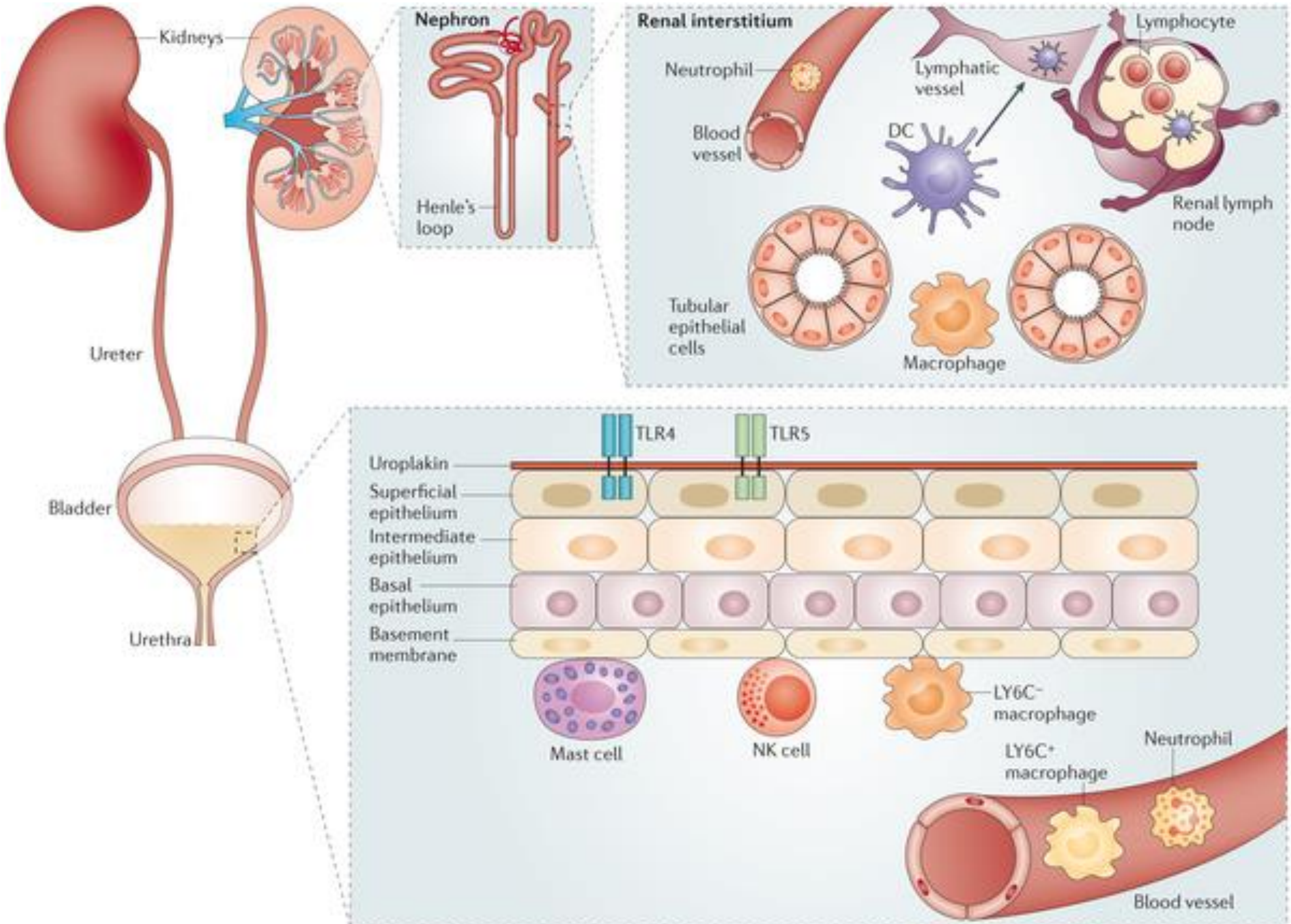
Assymetric and symmetric stem cell divisions in tissue repair



STEM CELLS



a Homeostasis
Enteroendocrine cell
Absorptive enterocyte
Tuft cell
Goblet cell
Paneth cell
Absorptive progenitor
DLL1⁺ secretory progenitor
LGR5⁺ LRC paneth cell progenitor
+4 stem cell
DLL1⁺
LRC
+4 stem cell
LGR5⁺ CBC stem cell
CBC stem cell
Pericryptal cell
b Injury
c Regeneration
Activation of 'reserve' stem cells (+4 stem cells) to regenerate LGR5⁺ stem cell pool
d Regeneration
Niche-driven conversion of DLL1⁺, LGR5⁺ LRC secretory precursors and other early TA cells to LGR5⁺ stem cells
• Loss of LGR5⁺ CBC stem cells
• Survival of LGR5⁺ LRCs and +4 stem cells
• Survival of TA cells (including DLL1+ progenitors)
• Maintenance of niche
Nature Reviews | Molecular Cell Biology



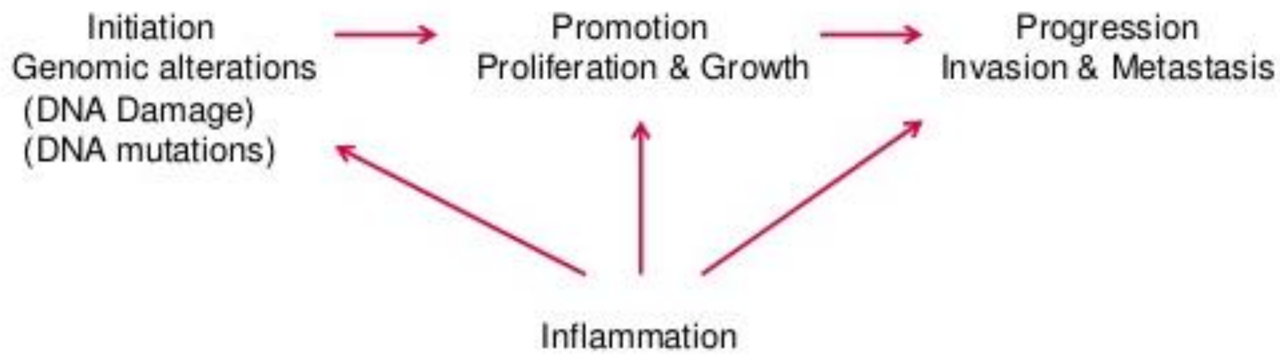
Nature Reviews | Immunology

Inflammation and Cancer

Link between cancer and inflammation

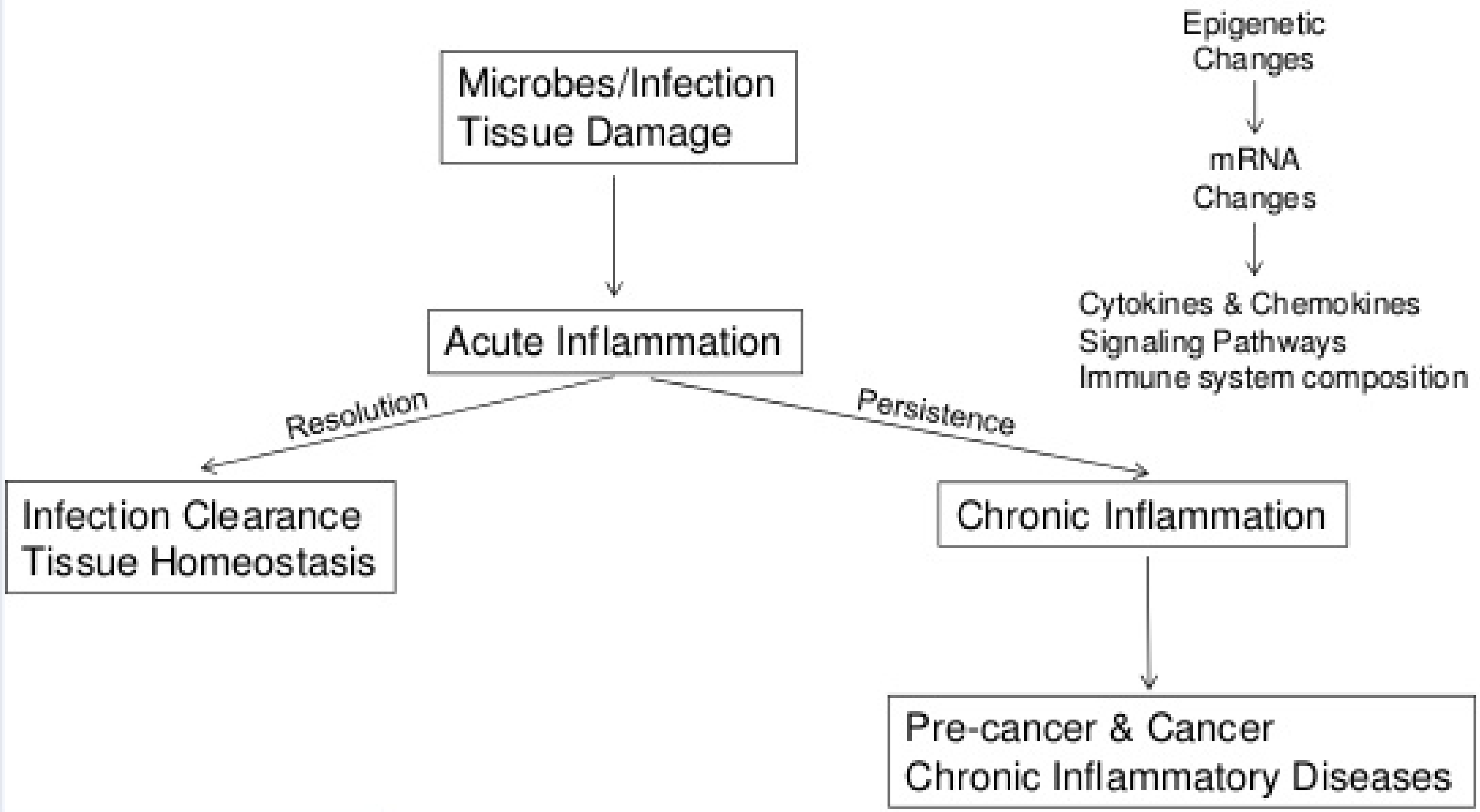
"Cancer is the result of uncontrolled growth of a given cell type that occurs together with the invasion of surrounding tissue and the spread of malignant cells."
Karin & Greten (2005) Nature Reviews Immunology (5): 749

Three Mechanistic phases



Link between cancer and inflammation

Definition: a protective tissue response to tissue damage or microbes, which serves to destroy, dilute, or wall off both the injurious agent and the injured tissues.

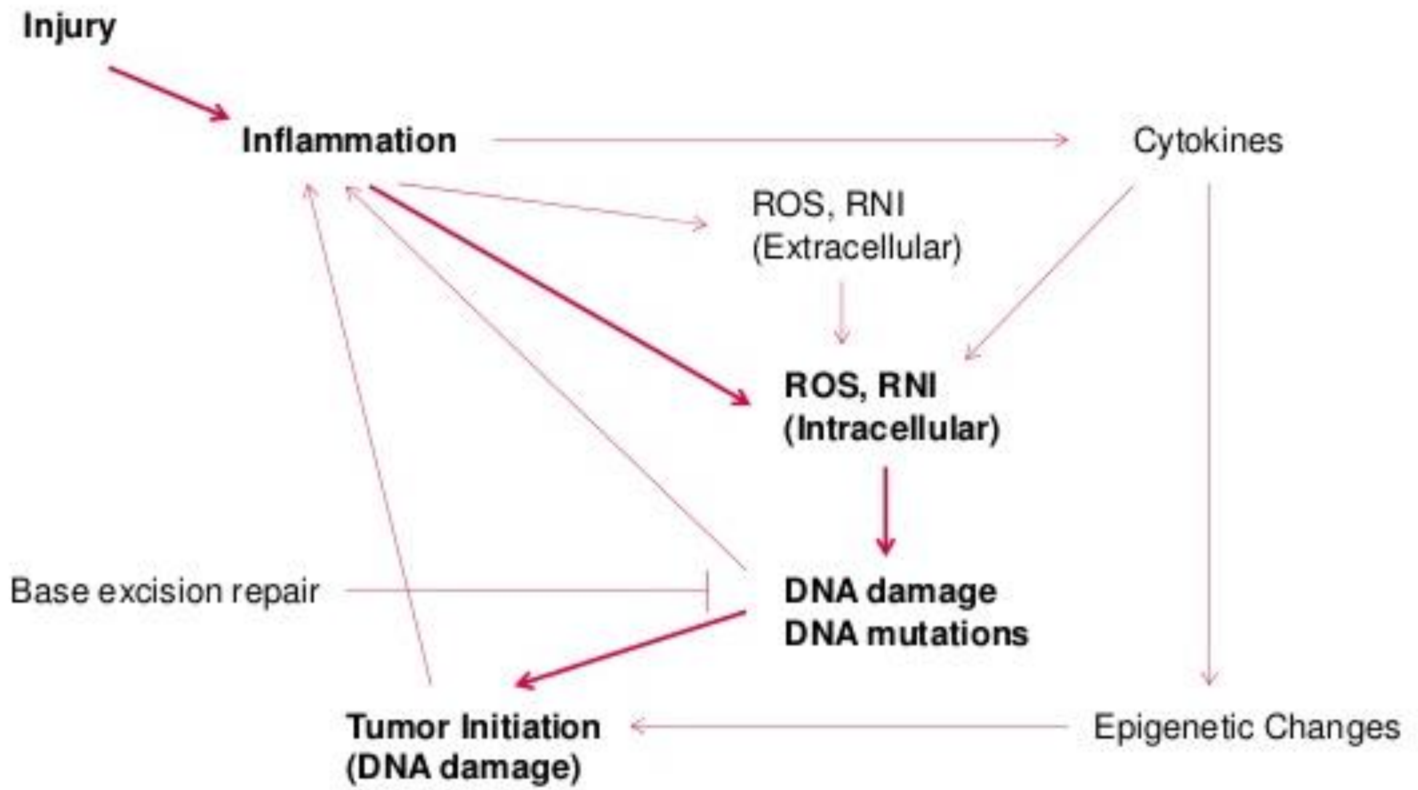


Link between cancer and inflammation

Bacteria, viruses, chemicals, chronic inflammation

Malignancy	Inflammatory Stimulus
Bladder cancer	Schistosomiasis
Gastric lymphoma	H. pylori-induced gastritis
MALT lymphoma	H. pylori
Hepatocellular carcinoma	HBV, HCV
Kaposi's sarcoma	HHV8
Bronchial carcinoma	Silica, Asbestos
Mesothelioma	Asbestos
Ovarian cancer	Endometriosis
Colorectal cancer	IBD
Esophageal cancer	Barrett's metaplasia
Papillary thyroid carcinoma	Thyroiditis
Prostate cancer	Prostatitis

INFLAMMATION AND CANCER INITIATION



Adapted from Grivennikov et al, Cell 2010

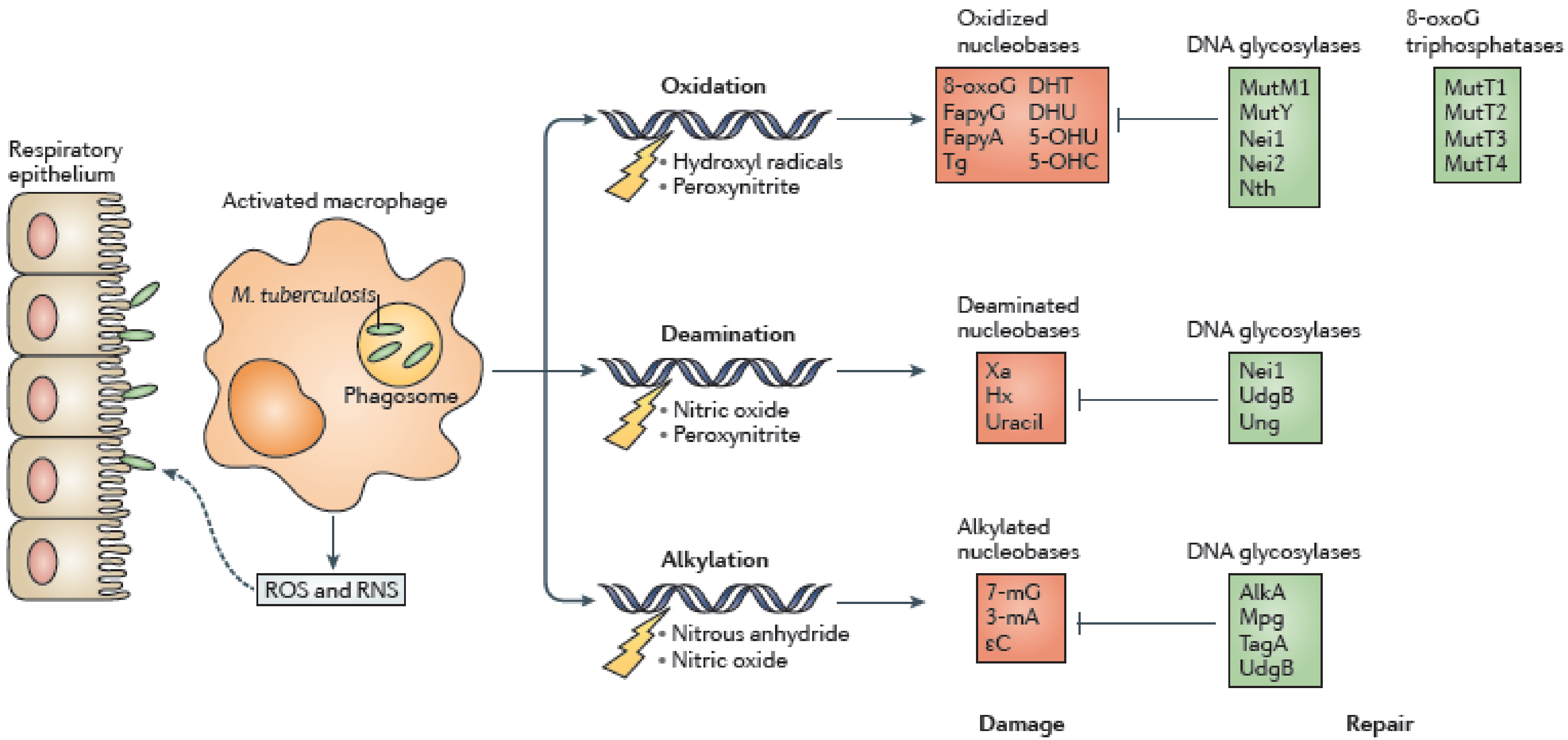


Figure 2 | **Redundancy in the *Mycobacterium tuberculosis* BER pathway.** *M. tuberculosis* colonizes the respiratory epithelium and is subsequently phagocytized by infiltrating alveolar macrophages and other phagocytic cells. Inside the phagosome of activated macrophages, the bacterium is exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in many modifications of nucleobases through oxidation (via hydroxyl radicals and peroxynitrite), deamination (via nitric oxide and peroxynitrite) and alkylation (via nitrous anhydride and nitric oxide) reactions. The most frequently encountered oxidized nucleobases include 7,8-dihydro-8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA), thymine glycol (Tg), 5,6-dihydrothymine (DHT), 5,6-dihydrouracil (DHU), 5-hydroxyuracil (5-OHU) and 5-hydroxycytosine (5-OHC). The most common deaminated nucleobases include xanthine (Xa), hypoxanthine (Hx) and uracil, and the most frequent alkylated nucleobases are 7-methylguanine (7-mG), 3-methyladenine (3-mA) and ethenocytosine (εC). These single-nucleotide lesions are generally repaired by base excision repair (BER), and *M. tuberculosis* has evolved remarkable functional redundancy in this pathway. It expresses a large number of DNA glycosylases that recognize and excise a wide variety of damaged nucleobases. For example, *M. tuberculosis* expresses MutY, which excises adenine, guanine and thymine following their mispairing with 8-oxoG. *M. tuberculosis* also harbours four genes encoding potential 8-oxoG triphosphatase (MutT) orthologues, and it expresses the two apurinic or apyrimidinic (AP) endonucleases XthA and End for the repair of AP sites (not shown).

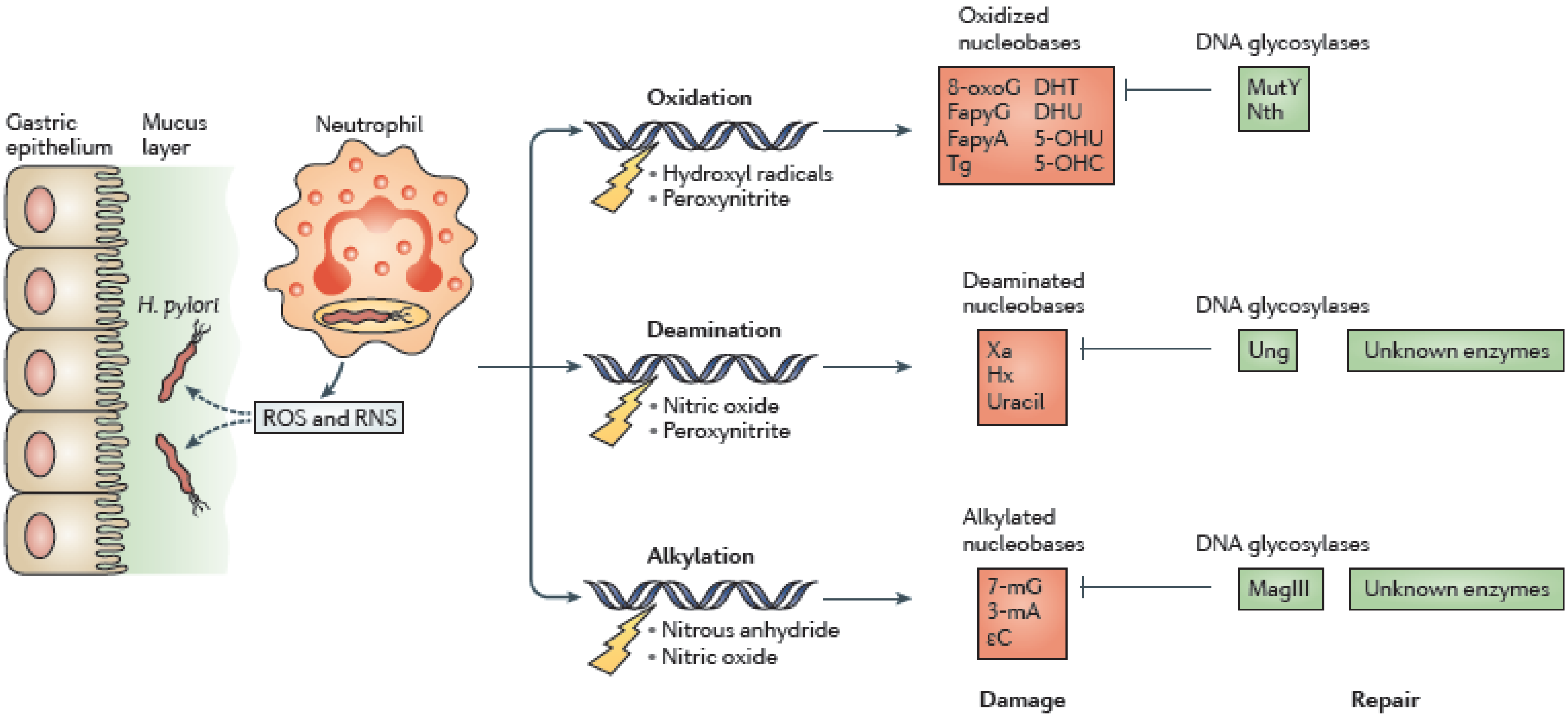


Figure 3 | **Functional specialization of the *Helicobacter pylori* BER pathway.** *H. pylori* colonizes the gastric mucosa and can also be phagocytized by infiltrating neutrophils. Both intracellular and extracellular *H. pylori* are exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are generated by a strong 'respiratory burst' from neutrophils. ROS and RNS damage DNA by oxidation reactions (mediated by hydroxyl radicals and peroxynitrite), which generate 7,8-dihydro-8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA), thymine glycol (Tg), 5,6-dihydrothymine (DHT), 5,6-dihydrouracil (DHU), 5-hydroxyuracil (5-OHU) and 5-hydroxycytosine (5-OHC); deamination (mediated by nitric oxide and peroxynitrite), which generates xanthine (Xa), hypoxanthine (Hx) and uracil; and alkylation (mediated by nitrous anhydride and nitric oxide), which generates 7-methylguanine (7-mG), 3-methyladenine (3-mA) and ethenocytosine (εC). These single-nucleobase lesions are repaired by the base excision repair (BER) pathway. *H. pylori* has three DNA glycosylases (Nth, Ung and MagIII) that recognize and excise several of the most cytotoxic nucleobases. Interestingly, these DNA glycosylases do not recognize and excise nucleobase lesions that are mutagenic. However, *H. pylori* MutY excises adenine mispaired with 8-oxoG and thereby reduces the level of spontaneous mutations. Furthermore, *H. pylori* has the apurinic or apyrimidinic (AP) endonuclease XthA for the repair of AP sites (not shown).

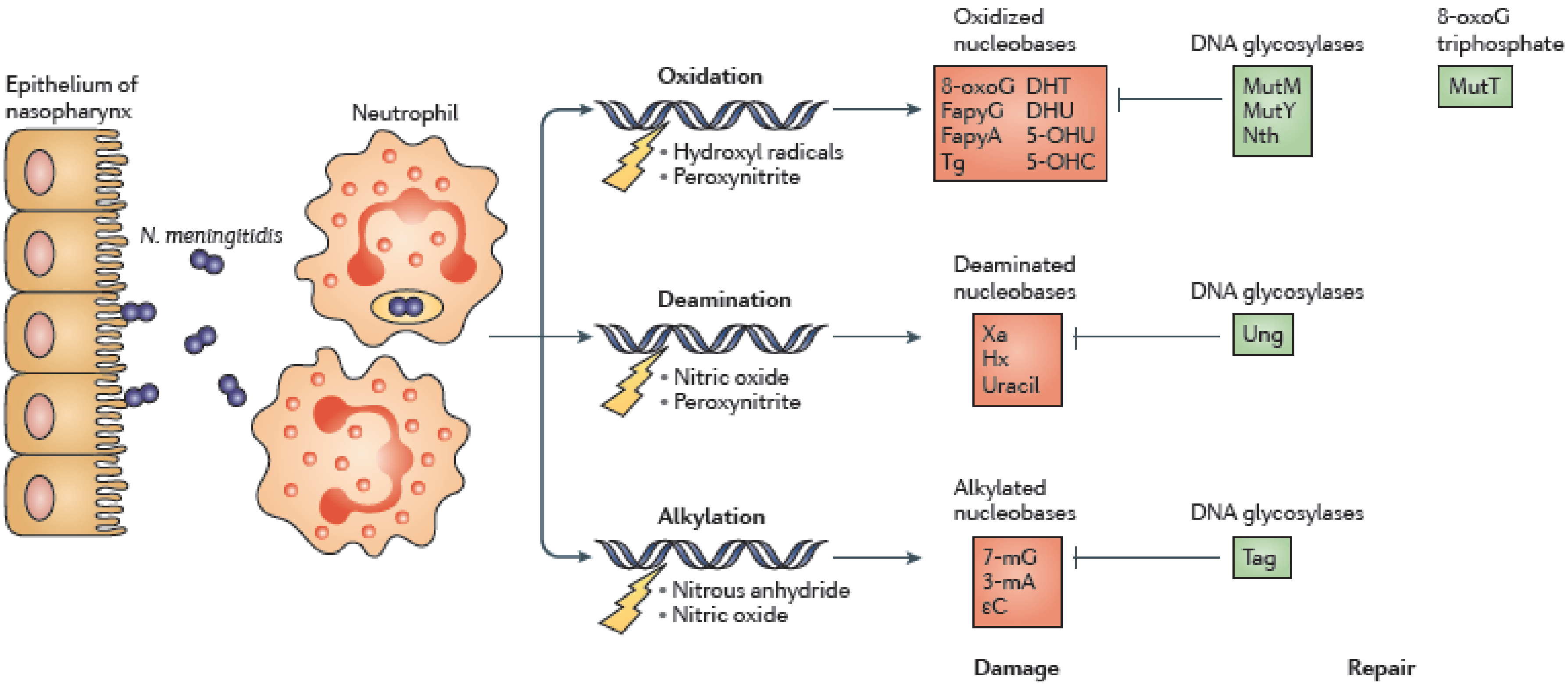
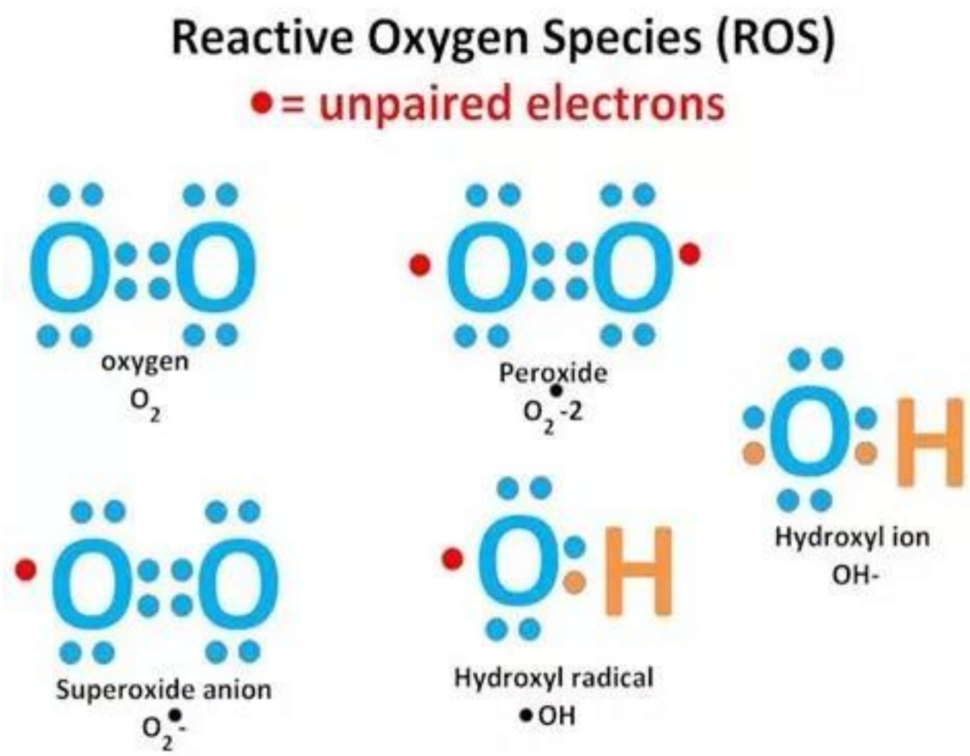


Figure 4 | **The BER network of *Neisseria meningitidis*.** *N. meningitidis* colonizes the human nasopharynx, where it is exposed to reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by atmospheric oxygen and by the 'respiratory burst' of phagocytes. Oxidized nucleobases include 7,8-dihydro-8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 4,6-diamino-5-formamidopyrimidine (FapyA), thymine glycol (Tg), 5,6-dihydrothymine (DHT), 5,6-dihydrouracil (DHU), 5-hydroxyuracil (5-OHU) and 5-hydroxycytosine (5-OHC). Deaminated bases include xanthine (Xa), hypoxanthine (Hx) and uracil; alkylated nucleobases include 7-methylguanine (7-mG), 3-methyladenine (3-mA) and ethenocytosine (εC). *N. meningitidis* has several DNA glycosylases with overlapping functions (such as MutM and Nth) that excise oxidized nucleobases. *N. meningitidis* harbours a putative uracil DNA glycosylase (Ung) for the removal of uracil (it is unknown whether this enzyme also removes the other deaminated bases Xa and Hx) and a putative 3-methyladenine DNA glycosylase (Tag) that removes 3-methyladenine (it is unknown whether this enzyme also removes the other alkylated bases 7-mG and εC). In addition, *N. meningitidis* MutY excises adenine, guanine and thymine mispaired with 8-oxoG; the bacterium contains a single 8-oxoG triphosphatase (MutT) homologue, which hydrolyses cellular 8-oxoGTP to 8-oxoGMP. Finally, meningococcal NApe is a true apurinic/apyrimidinic (AP) endonuclease, whereas NExo lacks AP endonuclease activity but instead is a specialized 3′-phosphatase (not shown).



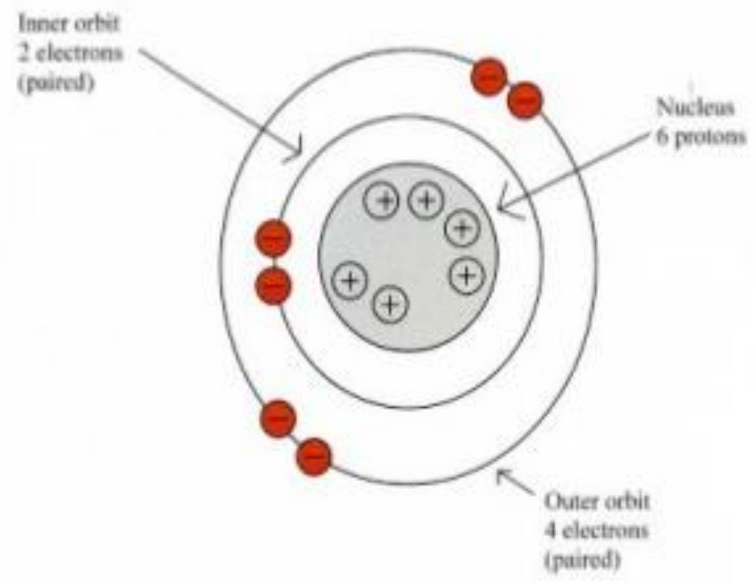
Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include peroxides, superoxide, hydroxyl radical, and singlet oxygen.

In a biological context, ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis.

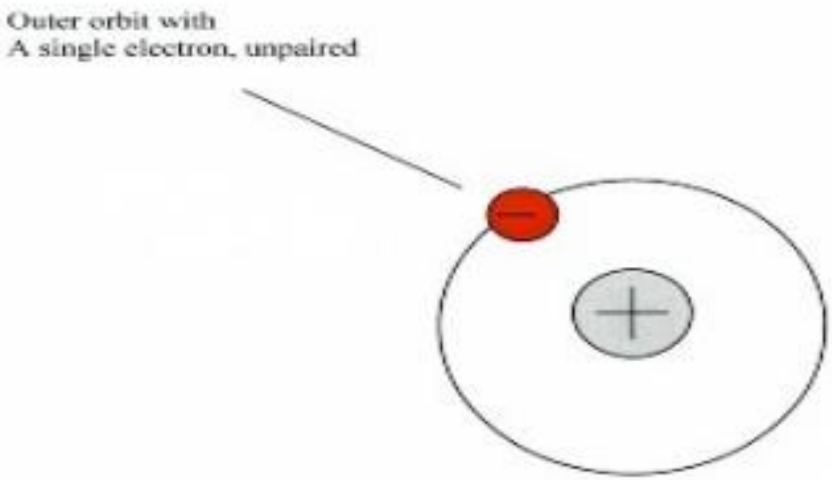
Free radicals

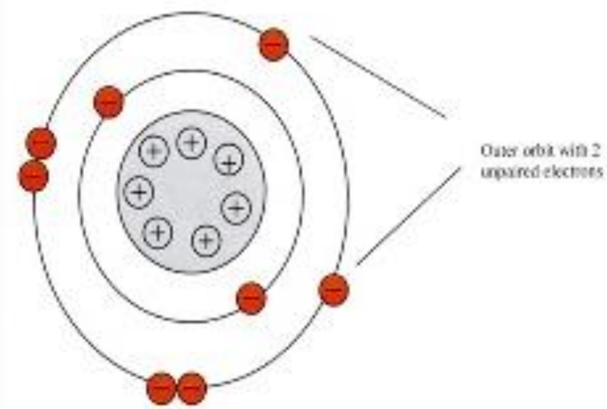
- Radicals are atoms, molecules, or ions with unpaired electrons in outer shell configuration.
- Free radicals may have positive, negative or zero charge.
  - Even though have unpaired electrons, by convention, metals and their ions or complexes with unpaired electrons are not radicals.
- Unpaired electrons cause radicals to be highly reactive.

Electron pairing in the outermost orbit indicates stability of the atom. To maintain stability, each electron in the outer orbit must be paired with another electron.



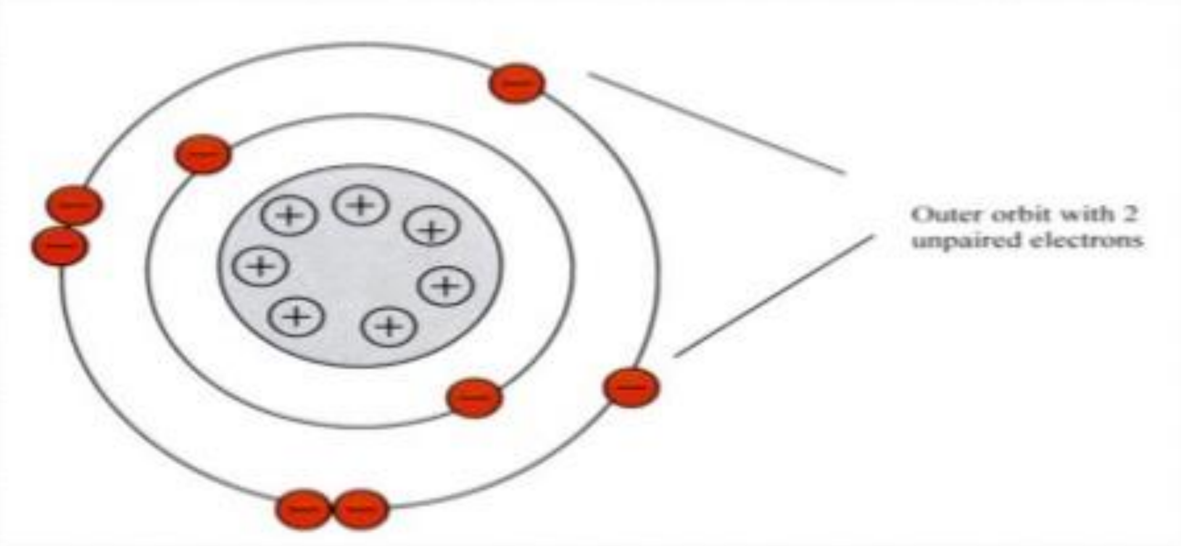
A free radical is simply an atom with one or more unpaired electrons in its outer orbit.





Oxygen as a free radical

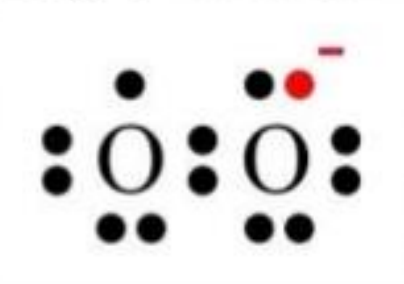
- Probably the most well-known free radical, oxygen is the basis for development of most free radicals in the body. Inherently, oxygen is an unstable molecule.



- During metabolism, the O_2 molecule splits and energy is released. To regain stability, the free single oxygen atom (oxygen free radical) seeks out or steals electrons from other available sources. This may result in a bond with dangerous properties:

- If oxygen accepts one electron, it becomes *superoxide anion radical.*

- If oxygen accepts two electrons, it produces *peroxide.*

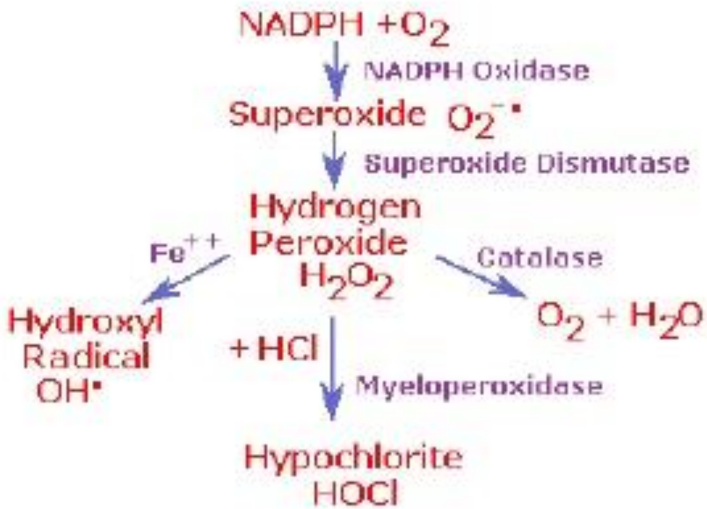


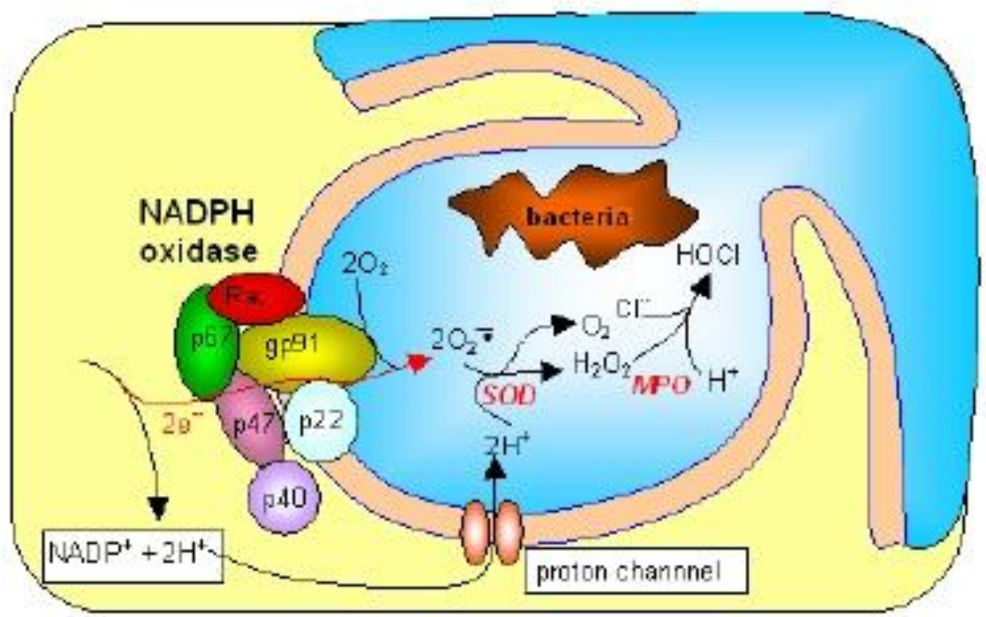
- Although the superoxide radical isn’t very powerful, it can easily donate an electron to a nearby iron atom to produce the hydroxyl radical (OH*), one of the most potent biological free radicals. OH* can react with almost any molecule to cause oxidative stress and damage. These oxygen free radicals also are called reactive oxygen species (ROS).

Some characteristics of ROS

ROS	Symbol	Half-life	Properties
Superoxide radical	$O_2^{\cdot-}$	10^{-6} s	poor oxidant
Hydroperoxyl radical	$HO_2^{\cdot}$		stronger oxidant than $O_2^{\cdot-}$
Hydrogen peroxide	H_2O_2	minúty	oxidant, diffuses across membranes
Hydroxyl radical	$OH^{\cdot}$	10^{-9} s	extremely reactive, diffuses only to very low distance
Alkoxyl radical	$LO^{\cdot}$	10^{-6} s	less reactive than $OH^{\cdot}$, but more than $ROO^{\cdot}$
Peroxyl radical	$LOO^{\cdot}$	10^{-2} s	weak oxidant, high diffusability
Singlet oxygen	$^1O_2$	10^{-6} s	powerful oxidizing agent

Formation of ROS and peroxynitrous acid in phagocytic vacuole of phagocytes

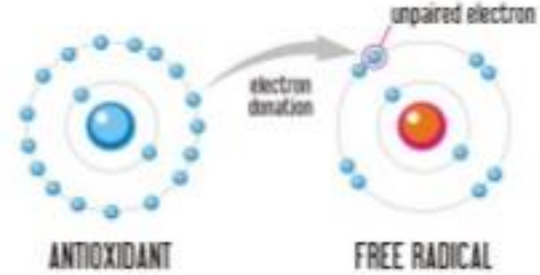




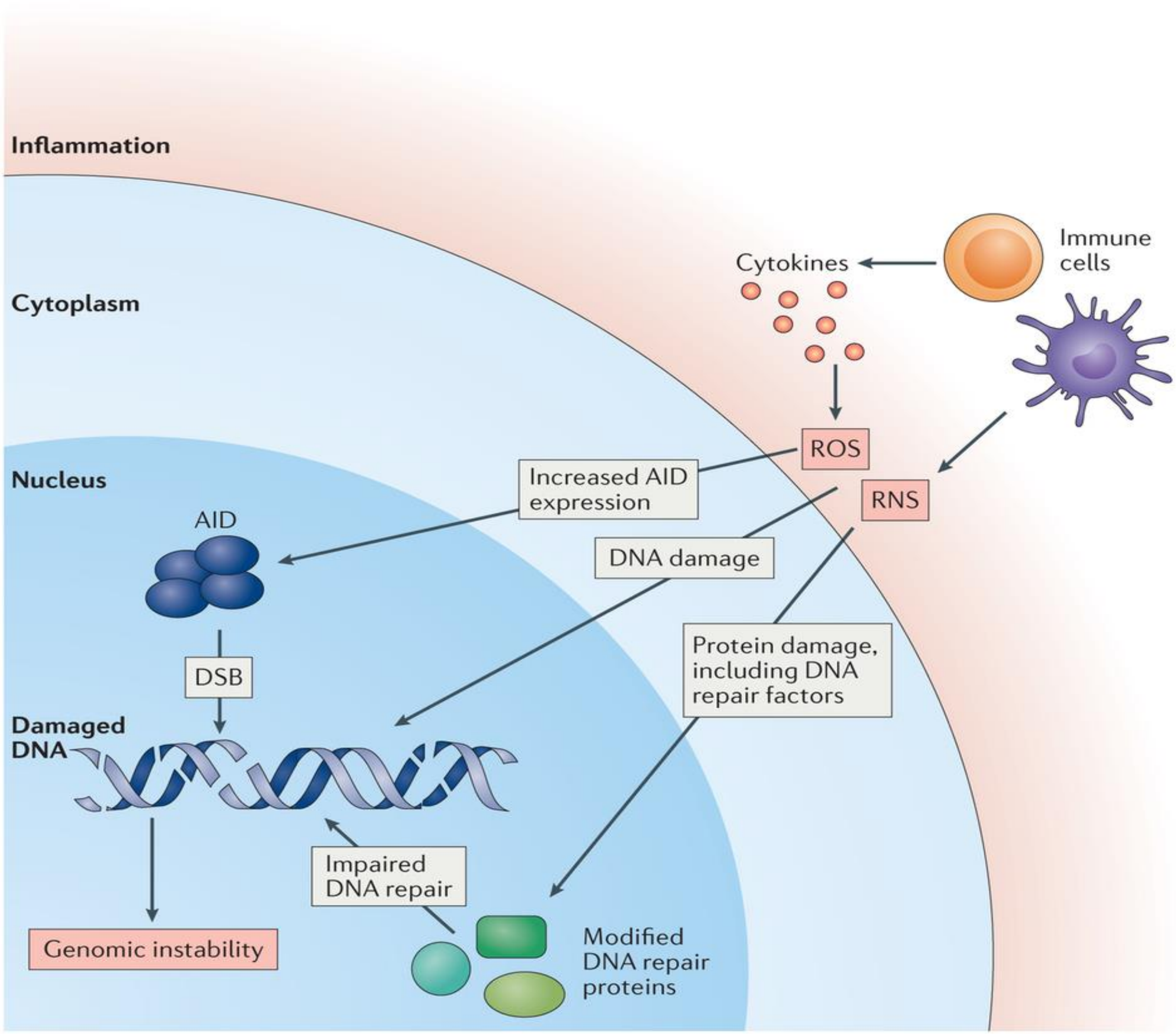
SOD – superoxid dismutase
MPO - myeloperoxidase

Antioxidants

- Non-enzymatic antioxidants
  - Alpha tocopherol (vitamin E)
  - Ascorbic acid (vitamin C)
  - Beta carotene



- Antioxidant enzymes
  - Superoxide dismutase (SOD) $O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \rightarrow O_2 + H_2O_2$
  - Catalase (CAT) $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
  - Glutathione peroxidase (GPx) $2\ G\text{-}SH + R\text{-}O\text{-}OH \rightarrow G\text{-}S\text{–}S\text{-}G + R\text{-}OH + H_2O$



Nature Reviews | Rheumatology

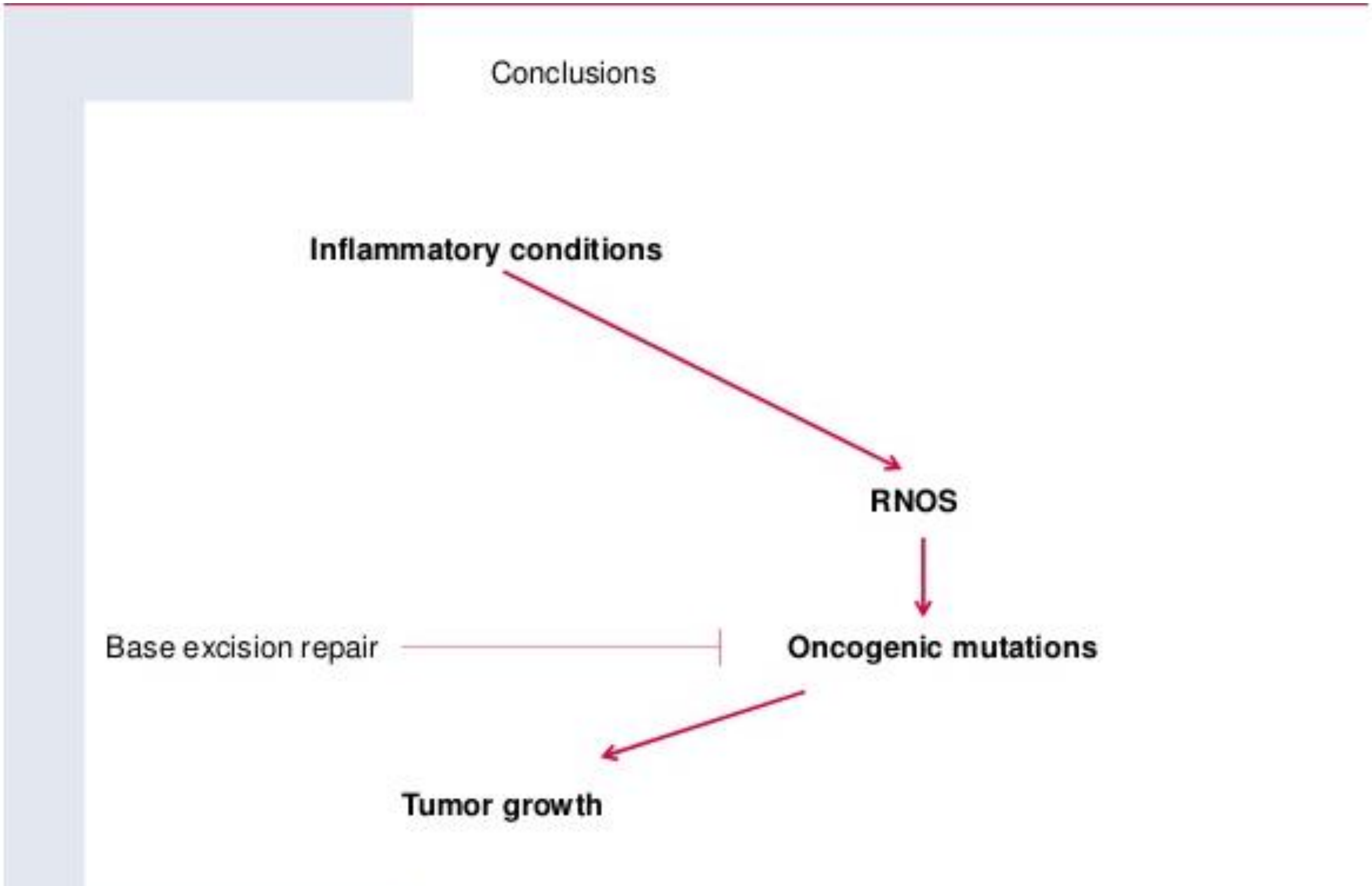
DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice

Lisiane B. Meira,[1] James M. Bugni,[1] Stephanie L. Green,[1] Chung-Wei Lee,[1,2] Bo Pang,[1]
Diana Borenshtein,[1,2] Barry H. Rickman,[1,2] Arlin B. Rogers,[1,2] Catherine A. Moroski-Erkul,[1]
Jose L. McFaline,[1] David B. Schauer,[1,2] Peter C. Dedon,[1] James G. Fox,[1,2] and Leona D. Samson[1]

[1]Department of Biological Engineering and Center for Environmental Health Sciences and [2]Division of Comparative Medicine,
Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

The Journal of Clinical Investigation http://www.jci.org Volume 118 Number 7 July 2008

TUMOR INITIATION



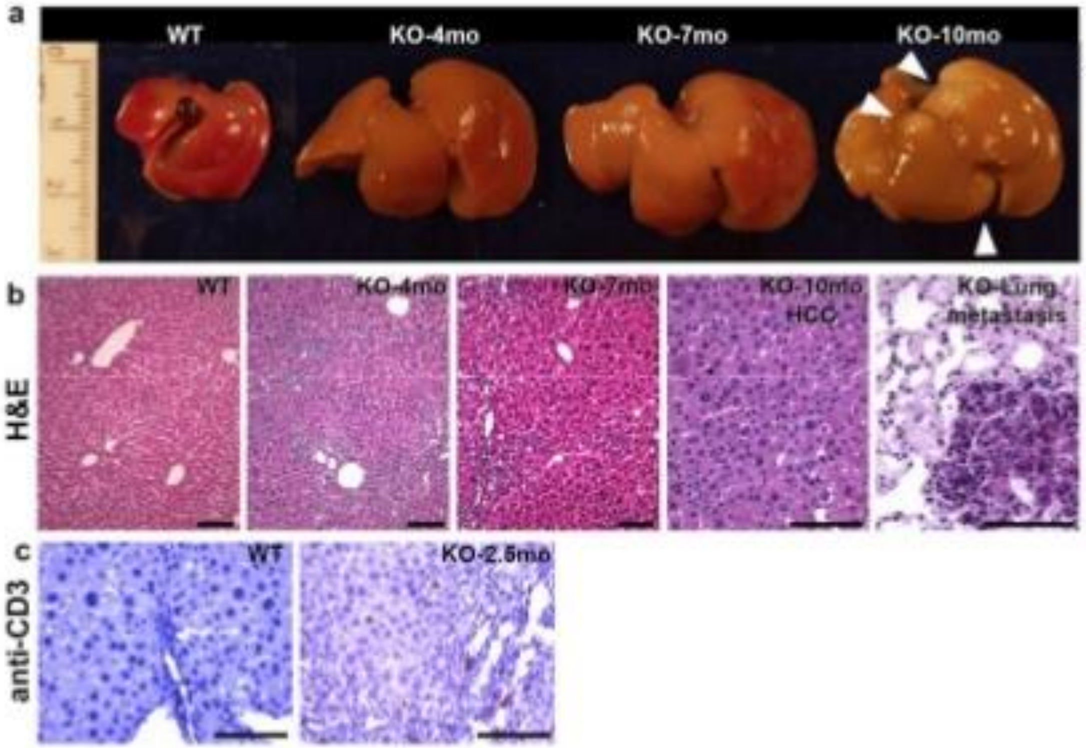
TUMOR PROGRESSION

NF-κB functions as a tumour promoter in inflammation-associated cancer

Eli Pikarsky[1*], Rinnat M. Porat[1*], Ilan Stein[1,2*], Rinat Abramovitch[3], Sharon Amit[2], Shafika Kasem[1], Elena Gutkovich-Pyest[2], Simcha Urieli-Shoval[4], Eithan Galun[3] & Yinon Ben-Neriah[2]

NATURE | VOL 431 | 23 SEPTEMBER 2004 | www.nature.com/nature

Mdr2-KO mice develop hepatocellular carcinoma on the background of chronic hepatitis (inflammatory condition)



Arrowheads indicate tumors

H&E staining

TUMOR PROGRESSION AND METASTASIS

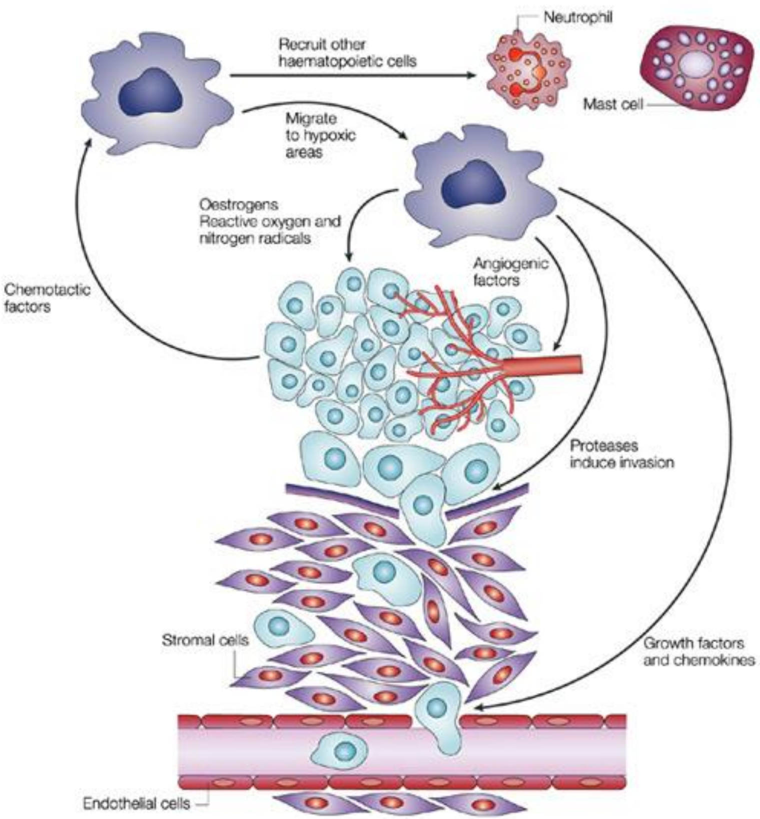
Colony-stimulating Factor 1 Promotes Progression of Mammary Tumors to Malignancy

By Elaine Y. Lin,[*] Andrew V. Nguyen,[*] Robert G. Russell,[‡]
and Jeffrey W. Pollard[*]

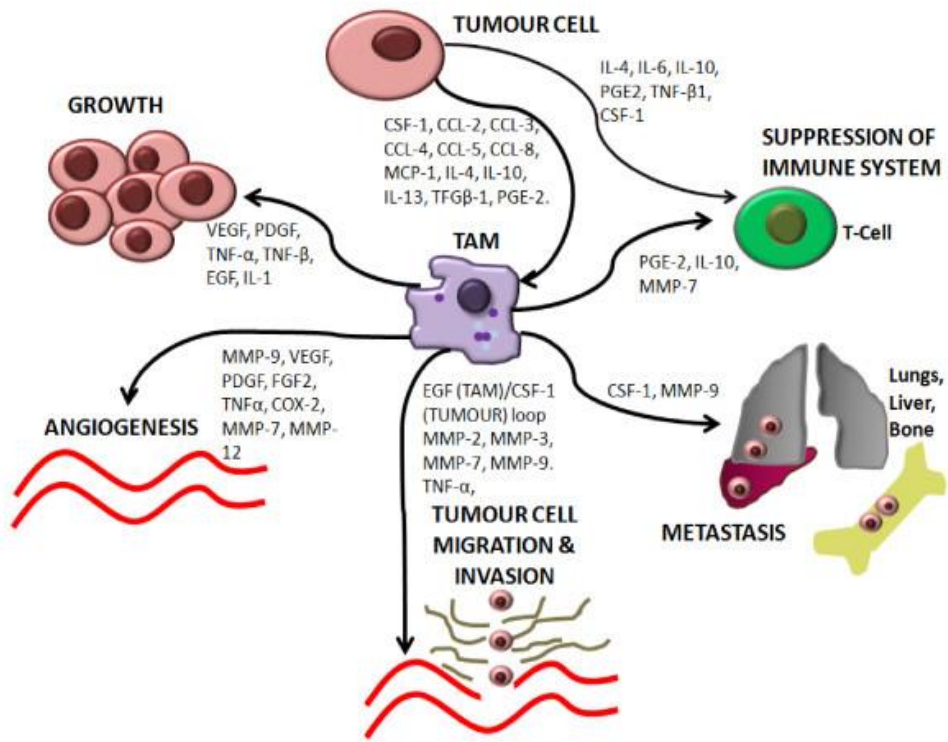
J. Exp. Med. © The Rockefeller University Press • 0022-1007/2001/03/727/13 $5.00
Volume 193, Number 6, March 19, 2001 727–739
http://www.jem.org/cgi/content/full/193/6/727

TAMs and CSF-1

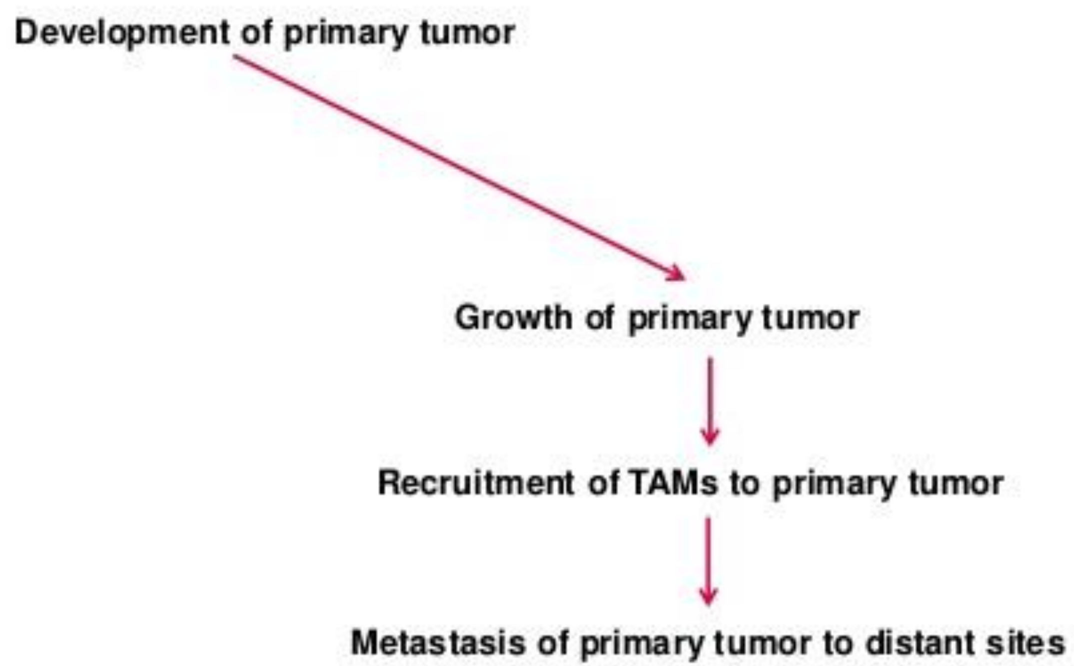
- Tumor-associated macrophages (TAMs) populate most solid tumors.
- TAMs are associated with poor prognosis.
- Colony-stimulating factor (CSF-1) is a regulator of the proliferation, differentiation, and survival of macrophages.
- CSF-1 expression is prevalent in invasive tumor cells.



Nature Reviews | Cancer



INFLAMMATION AND METASTASIS



How do bacteria contribute to cancer?

Inflammation

Toxins
(bacterial genotoxin)

Mechanisms by which the bacterial microbiome modulates carcinogenesis.

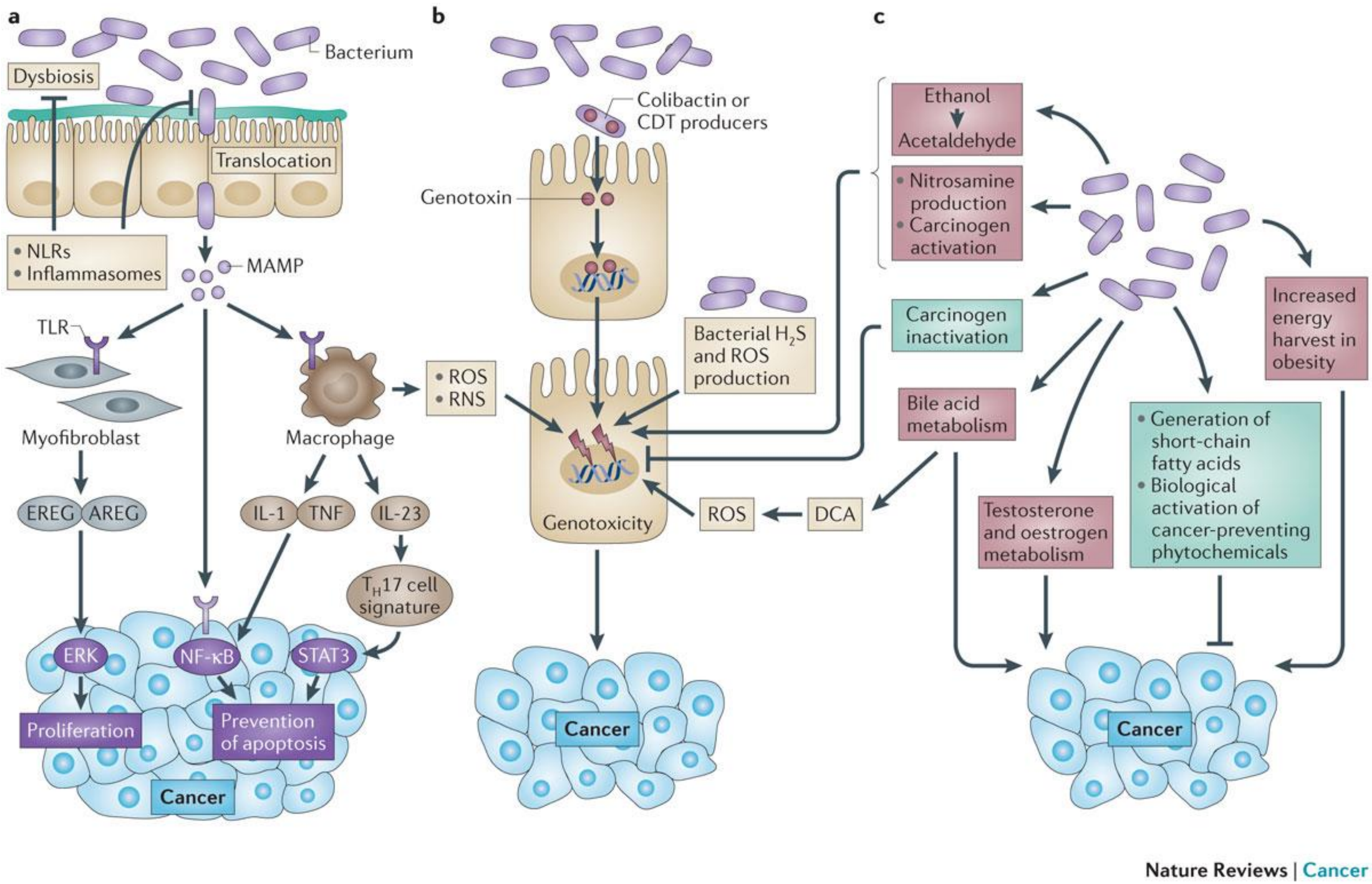


Figure 2 | **Mechanisms by which the bacterial microbiome modulates carcinogenesis.** The bacterial microbiome promotes carcinogenesis through several mechanisms. **a** | Changes in the microbiome and host defences may favour increased bacterial translocation, leading to increased inflammation, which is mediated by microorganism-associated molecular patterns (MAMPs) that activate Toll-like receptors (TLRs) in several cell types, including macrophages, myofibroblasts, epithelial cells and tumour cells. These effects may occur locally or through long-distance effects in other organs. **b** | Genotoxic effects are mediated by bacterial genotoxins — such as colibactin and cytolethal distending toxin (CDT) — that, after being delivered to the nucleus of host cells, actively induce DNA damage in organs that are in direct contact with the microbiome, such as the gastrointestinal tract. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) released from inflammatory cells such as macrophages, as well as hydrogen sulphide (H_2S) from the bacterial microbiota, may also be genotoxic. **c** | Metabolic actions of the microbiome may result in the activation of genotoxins such as acetaldehyde, dietary nitrosamine and other carcinogens, in the metabolism of hormones such as oestrogen and testosterone, in the metabolism of bile acids and in alterations of energy harvest. The microbiota also mediates tumour suppressive effects (shown in green) through inactivation of carcinogens, through the generation of short-chain fatty acids such as butyrate and through the biological activation of cancer-preventing phytochemicals. Many of these tumorigenic and tumour-suppressive mediators exert both local and long-distance effects. AREG, amphiregulin; DCA, deoxycholic acid; EREG, epiregulin; IL, interleukin; NF-κB, nuclear factor-κB; NLR, NOD-like receptor; STAT3, signal transducer and activator of transcription 3; T_H17, T helper 17; TNF, tumour necrosis factor.

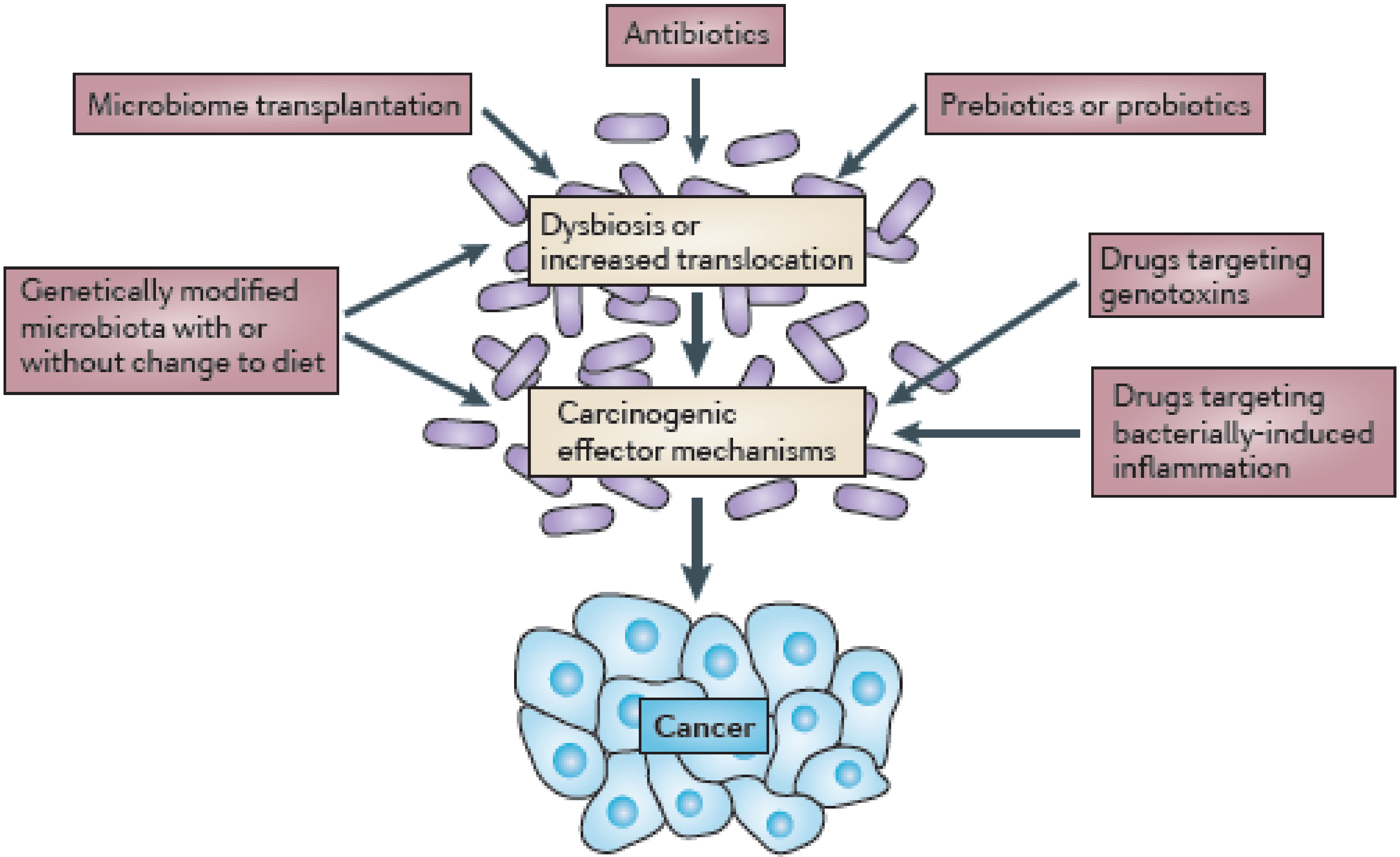


Figure 3 | **Targeting the bacterial microbiota for therapeutic modulation of carcinogenesis.** On the basis of the known contribution of the bacterial microbiota in experimental carcinogenesis, the approaches shown are conceivable for the prevention of human carcinogenesis.

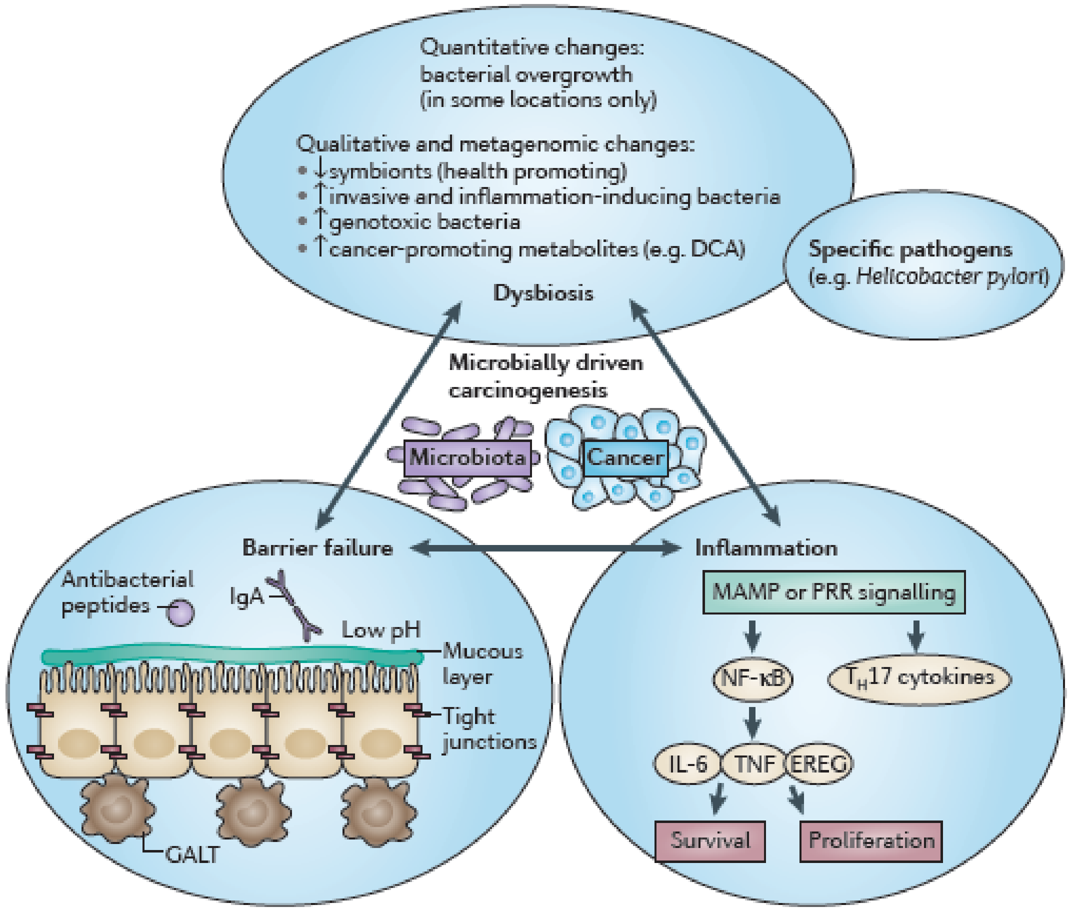


Figure 1 | **Mechanisms controlling host–microbiota interactions and associated failures implicated in cancer development.** A state of homeostasis and symbiotic relationships is maintained by the separation of microbial entities from the host through a multi-level barrier, by a eubiotic microbiome that actively suppresses pathobionts and that maintains a symbiotic relationship with the host, and by a state of low inflammation in the host. Perturbation of this balance leads to chain reactions that ultimately result in a cancer-promoting state with a failing barrier, inflammation and dysbiosis. This state includes qualitative and sometimes quantitative changes in the microbiota; failure of the barrier either physically (for example, at the level of tight junctions or at the mucous layer), or at the level of antibacterial defence systems — either those of epithelial cells or those of cells from the gut-associated lymphoid tissue (GALT); and increased inflammatory responses, which are often mediated by pattern recognition receptors (PRRs) and downstream cytokines that promote epithelial cell proliferation and survival. DCA, deoxycholic acid; EREG, epiregulin; IgA, immunoglobulin A; IL-6, interleukin-6; MAMP, microorganism-associated molecular pattern; NF-κB, nuclear factor-κB; T_H17, T helper 17; TNF, tumour necrosis factor.