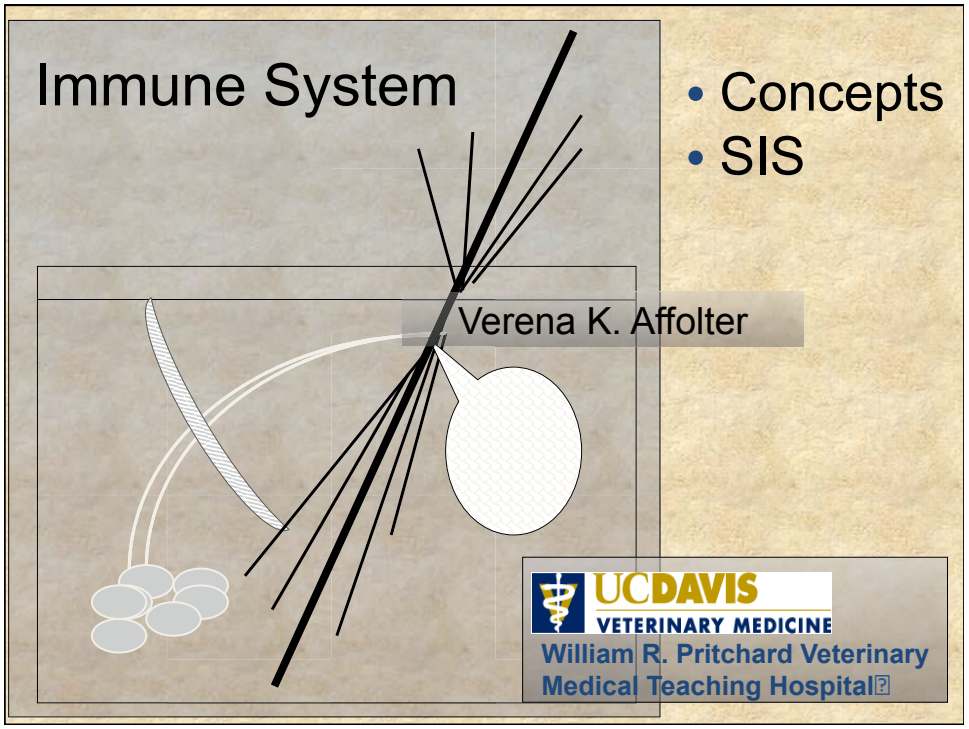


Innate immune system

- Immediate: non-leukocytic components
- Fast response: release of soluble components (non-leukocytic and leukocytic)
- Effector cells: phagocytosis (neutrophils, macrophages) NK cells
- No memory
- No individuality

Adaptive immune system

- Specificity
- Individuality
- Self-recognition
- Memory

antigen presentation
(MHC II, MHC II, CD1)
antigen recognition
V regions of the αβTCR
V regions of membrane bound IgM / IgD ("BCR") and soluble Ig
V regions of γδTCR

First response is delayed (priming)
Second response is faster (memory)

Ontogeny leukocytes

- T cells
- B cells
- NK cells
- Histiocytes
- Mast cells
- Neutrophils
- Eosinophils
- Basophils

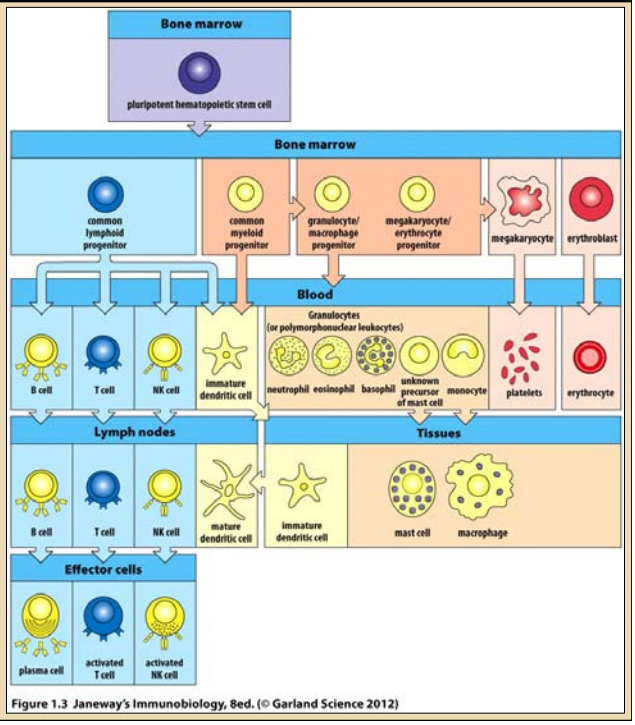
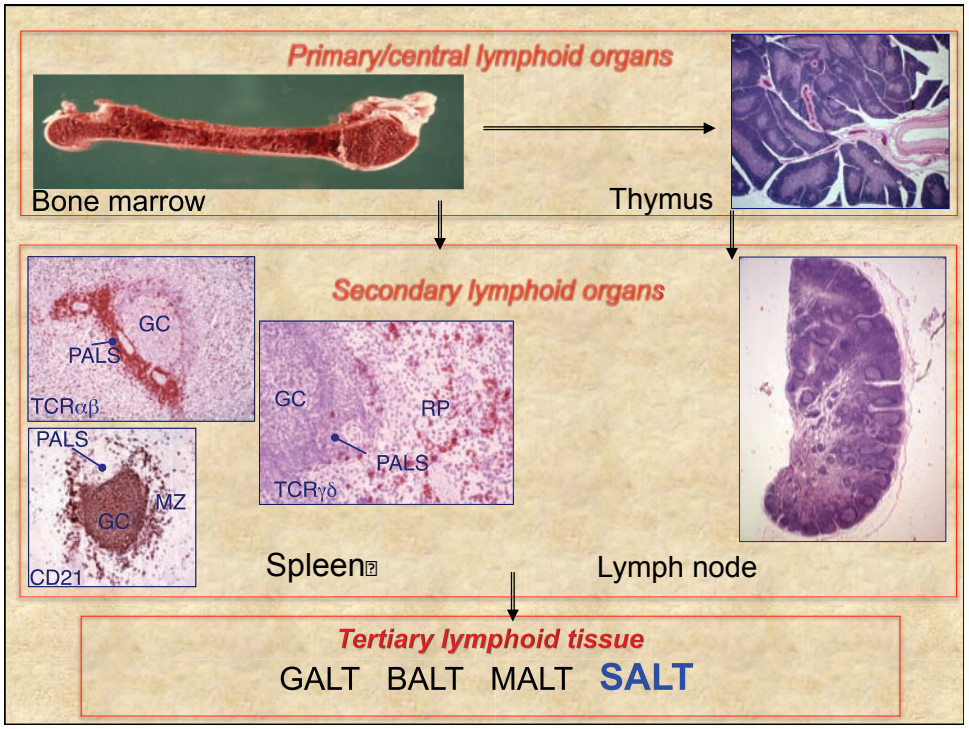
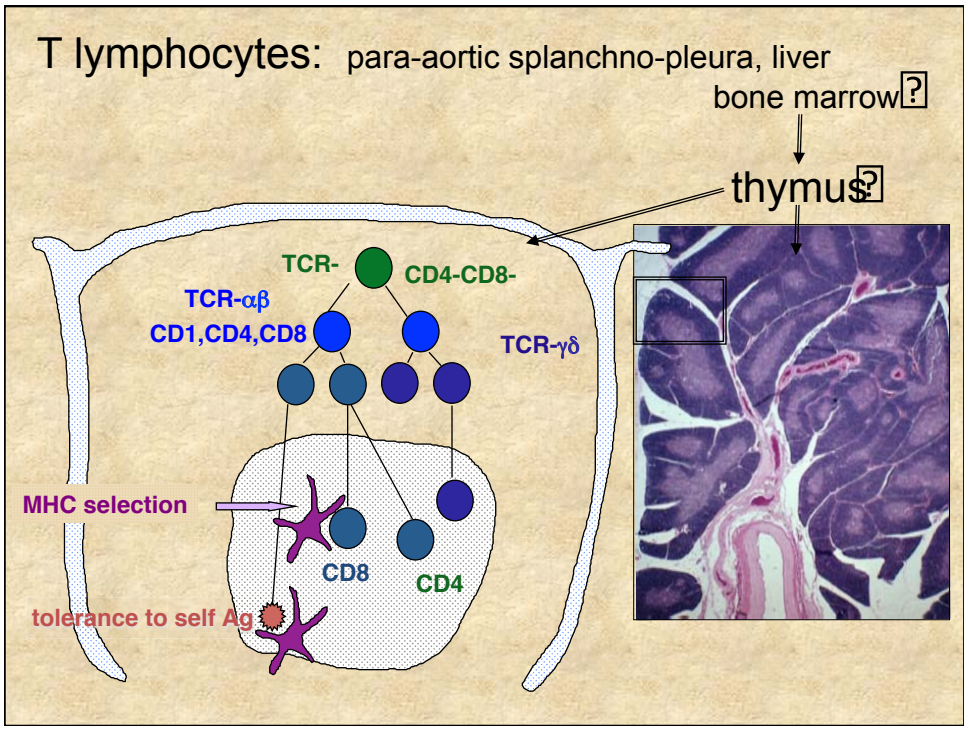


Figure 1.3 Janeway's Immunobiology, 8ed. (© Garland Science 2012)


Primary/central lymphoid organs
Bone marrow
Thymus
Secondary lymphoid organs
GC
PALS
TCRαβ
PALS
GC
MZ
CD21
GC
RP
PALS
TCRγδ
Spleen
Lymph node
Tertiary lymphoid tissue
GALT BALT MALT SALT


T lymphocytes: para-aortic splanchno-pleura, liver
bone marrow?
thymus?
TCR-
CD4-CD8-
TCR-αβ
CD1,CD4,CD8
TCR-γδ
MHC selection
CD8
CD4
tolerance to self Ag

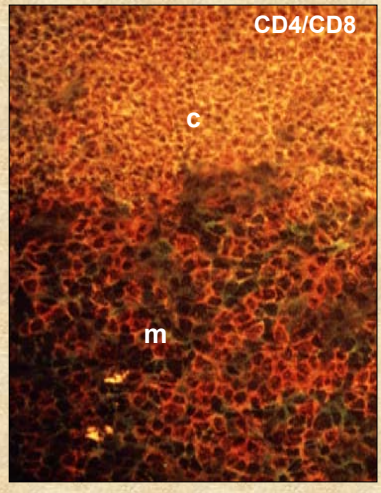
TCR rearrangement
- (RAG-1 and RAG-2)

Self MHC restriction
- positive selection

Self-tolerance
- negative selection

CCR7

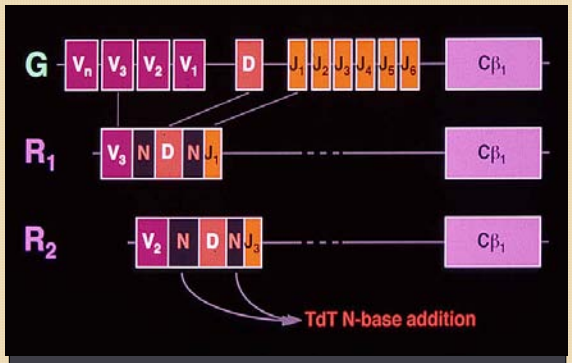
CCL19
CCL20



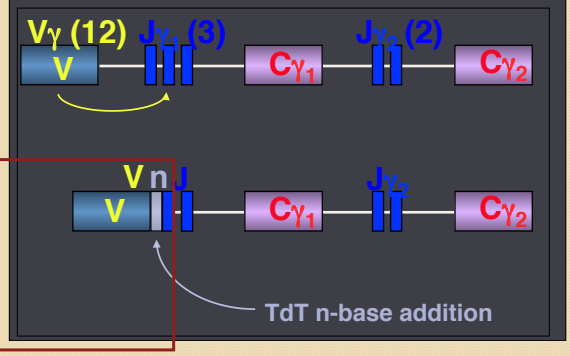
←Thymic epithelial: AIRE
(autoimmune regulatory protein)

TCR rearrangement

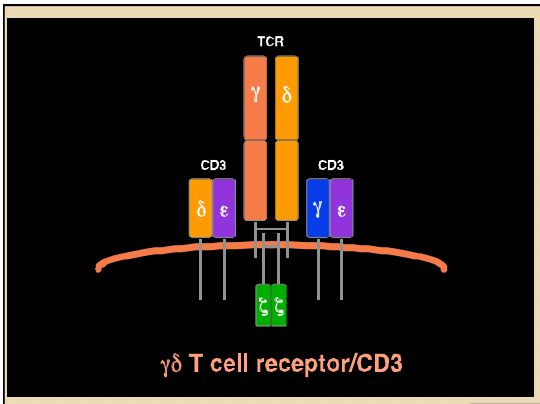
β-chain and δ-chain



α-chain and γ-chain



Clonality testing

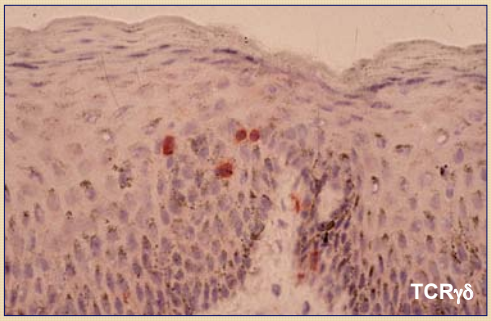


δγ-T cells

CD4$^-$CD8$^-$ or CD8$^+$

- red pulp spleen
- intestinal epithelium
- **epidermis**

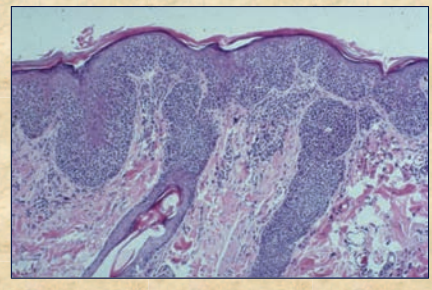
- 2% of cutaneous T cells



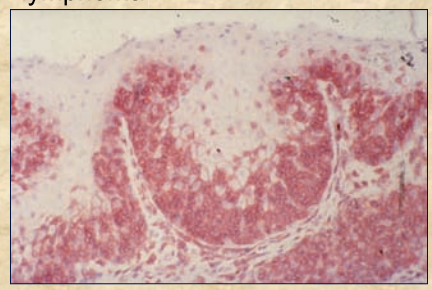
δγ-T cells

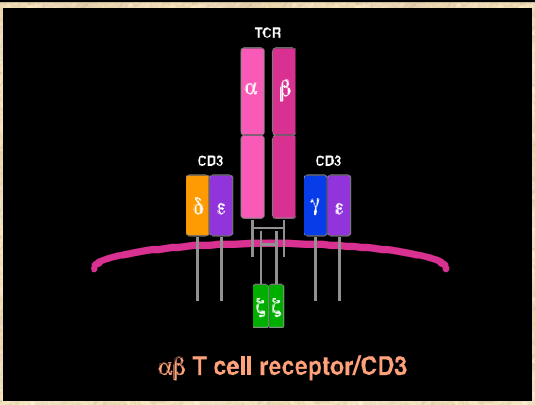
Function?
Little diversity in their TCR
Not interacting with MHC-peptide

Recognizing…..
heat shock proteins?
MHC Ib
phospholipids



Dog: Epitheliotropic T cell lymphoma

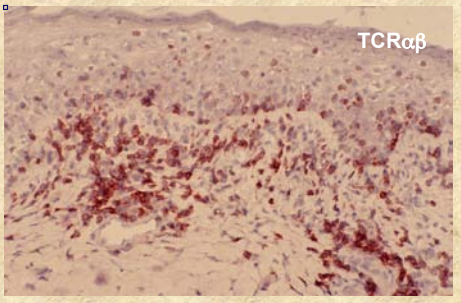




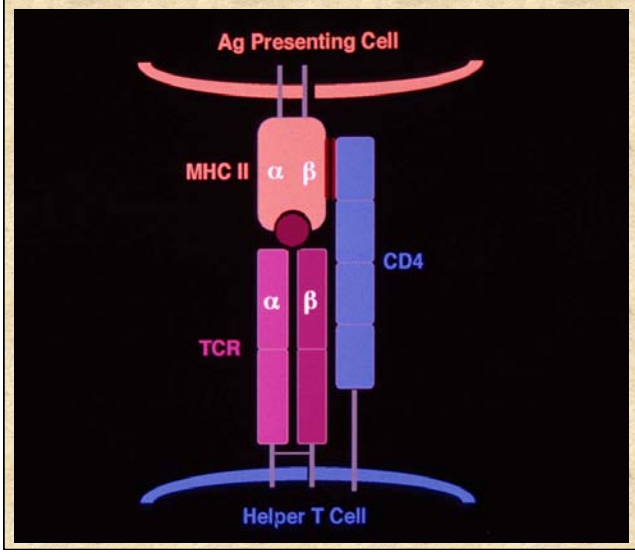
αβ-T cells
CD4⁺
CD8⁺

interact with MHC
(CD4⁺ - MHC II)
(CD8⁺ - MHC I)

- White pulp spleen
- Peripheral blood
- Paracortex Ln
- **Dermis** 98% of T cells; mostly memory cells (CD45RO⁺)
- **Epidermis** 2% of T cells



αβ-T cells - **CD4⁺**



- TREG
- TH17
- TH1 - helper cells
- TH2 - helper cells

- TGF-β, (IL-6)
- FoxP3
- Binding of APC - inhibits further binding to T_H cells
- Express CCR 7 to migrate to Ln

- IL-6
- Express ROR-γt
- Release IL-17: ⇑ IL-6, CXCL8, CXCL2, CSF
- Efficient amplifier of inflammation

- Prolonged chronic antigen challenge
- IL-12, IFN-γ, CCL3, CCL4, CCL5, TLR-APC:
- IL-12⇑
- Interaction with B cells: antibody production
- DTH
- Macrophage activity ⇑

- Mast cells, IL-4, IL-6, (absence of IL-12, IFN-γ)
- Interaction with B cells (CD40-40L): isotype switching

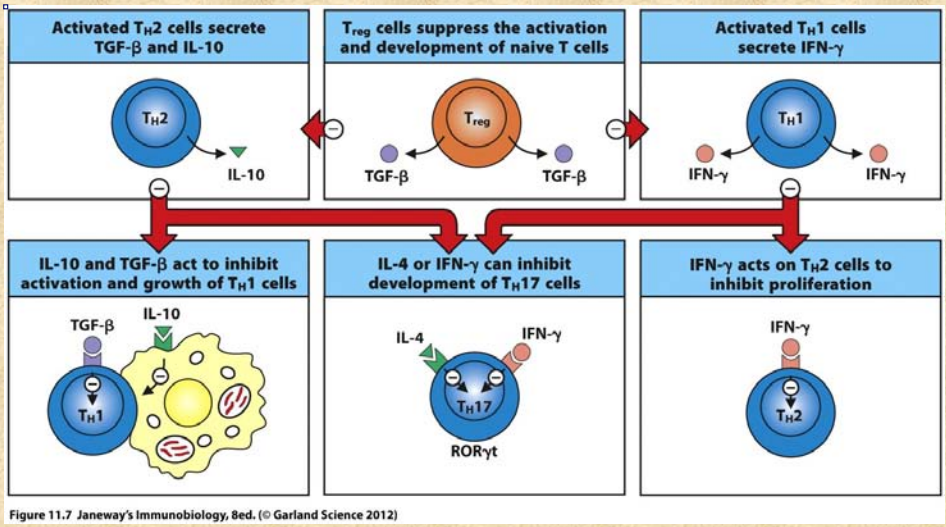
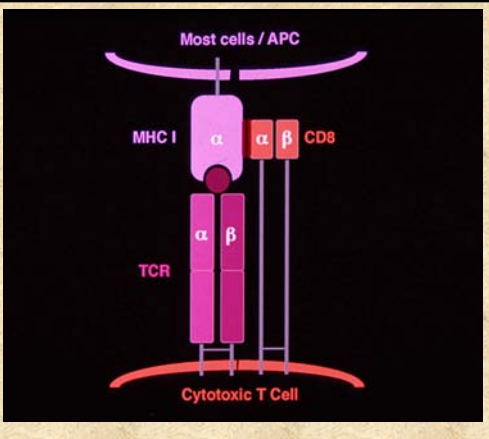


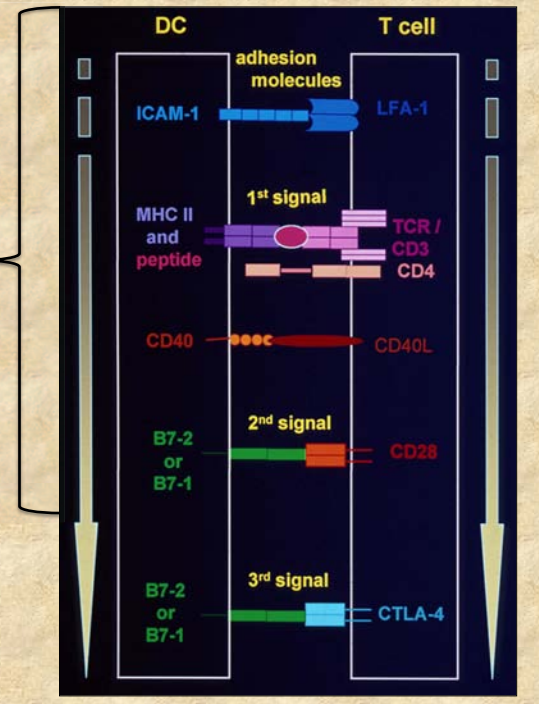
Figure 11.7 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

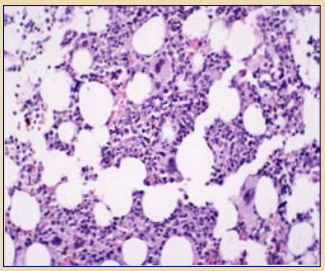
αβ-T cells - **CD8⁺**
CD8αα
CD8αβ



- <u>CD4 dependent</u>:
  Dual recognition of antigen by CD4 and CD8 cells
- <u>CD4 independent</u>:
  TLR-APC: APC activation of CD8 cell
- IL-12, IL-18
- Cell lysis: perforin, granzyme
- Apoptosis: Fas-FasL

T cells - activation

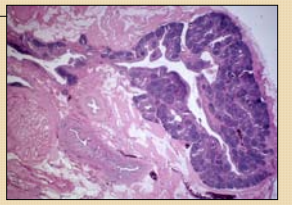


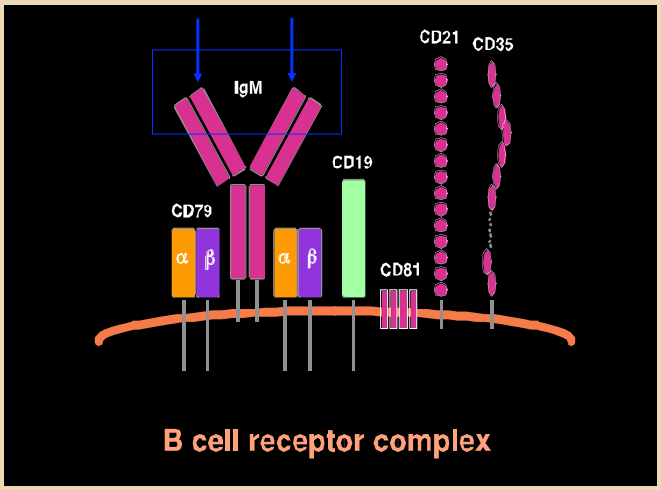


B cell development

Diversity

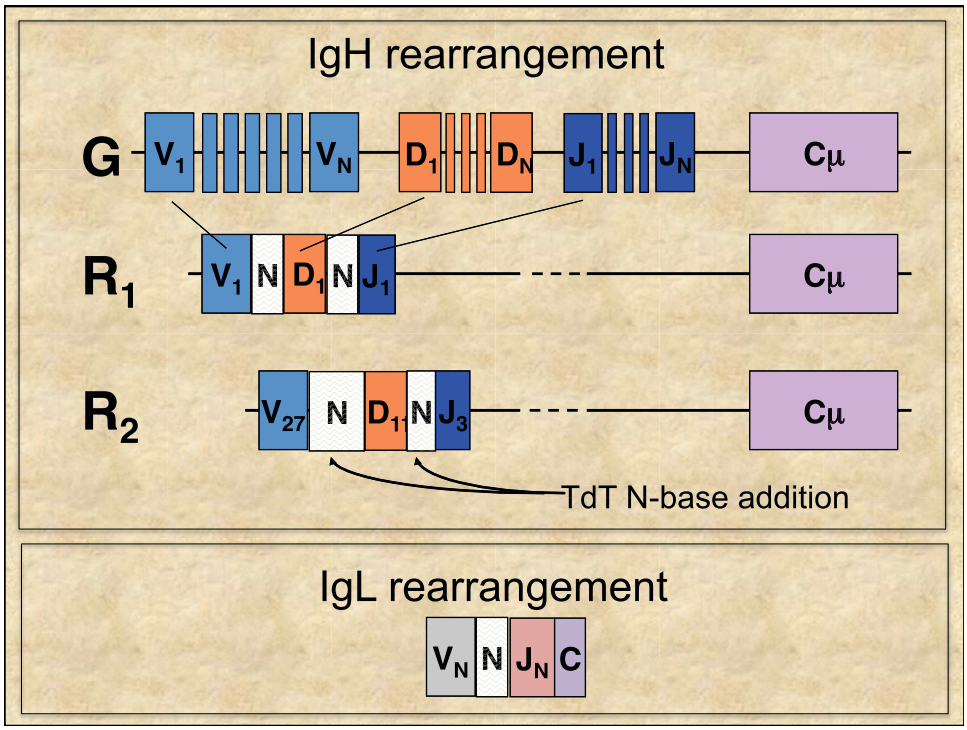
- BM: primates and mice
- Terminal ileal patch: ruminants (sheep)
- Bursa fabricii: birds
- Horse, dog, cat, swine?
- Somatic mutation - "affinity maturation"

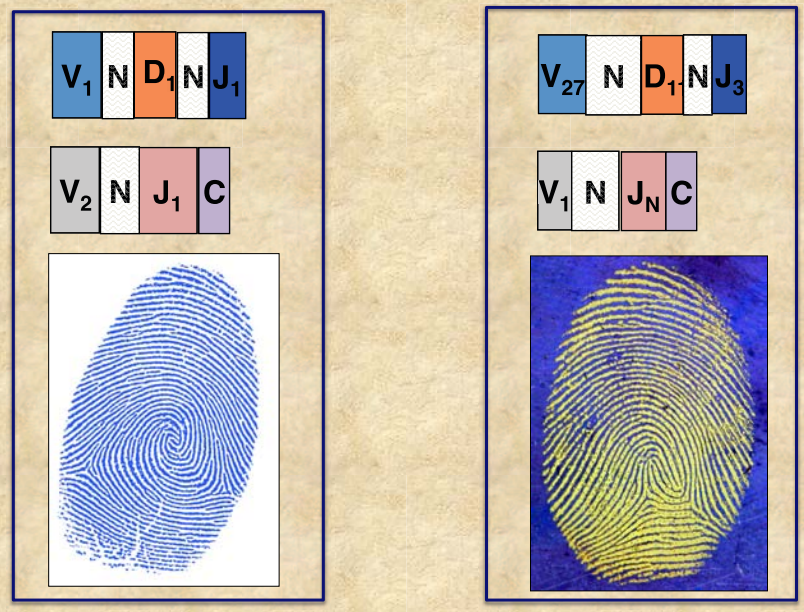




→BCR
IgM / IgD

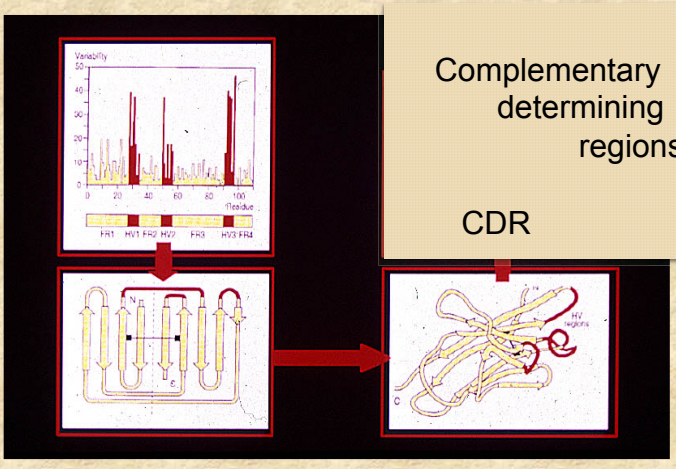
- Diversity: Rearrangement of the Ig gene (RAG-1, RAG-2):
  Ig heavy chain (IgH) - V_HDJ; Ig light chain - V_LJ
- Central tolerance: self antigens in high concentration (apoptosis)





Antigen binding site:
Unique sequences, lengths and combination

Antigen binding sites:
hypervariable regions in
V segments



Complementary
determining
regions

CDR

B cell populations:

light chain κ and λ

- dogs, cats, horses, cattle and sheep:
  - ✓ almost exclusively λ chain
- mice and rabbits:
  - ✓ more κ than λ
- humans, swine:
  - ✓ ratio 1:1.5 = κ : λ
- limited clonality testing using light chain

B cell sub-populations

B1-B cells

- Predominate in the fetus
- Self renewing in adults; peritoneal cavity
- CD5, IgM (little IgD)
- Sparse in peripheral lymphoid tissues
- Limited diversity - lack addition of nucleotides during Ig rearrangement
- Readily respond to bacterial polysacharides
- Lack specific interaction with memory T cells
- "Natural antibodies" (IgM)
- Low affinity (no hypermutation)
- Normal flora is considered "self"

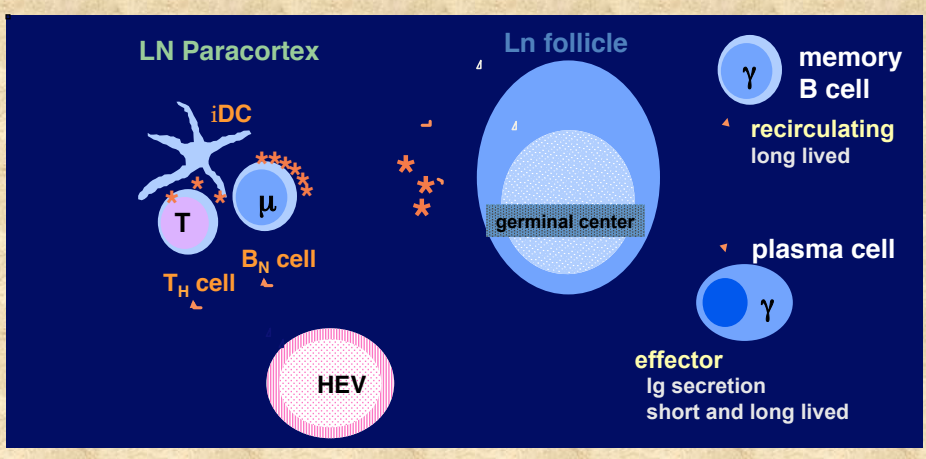
B cell sub-populations

B2 B cells

- continuously generated in the BM
- populate lymphoid tissues
- BCR: IgM (Cμ) and IgD (Cδ) (mice, human)
- Upon 1st antigen challenge: release of IgD and IgM

splenic white pulp ➔

B cell activation - plasma cell differentiation



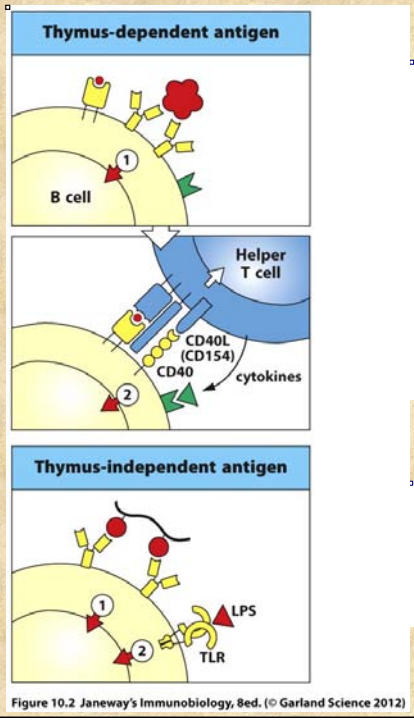


Figure 10.2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

T cell dependent
1. BCR – recognizes antigen
2. Antigen processing
3. B cell presents processed antigen to T cell
4. Activation through CD40-CD40L

T cell independent
1. BCR – recognizes antigen
2. TLR recognizes TLR-L

Somatic hypermutation

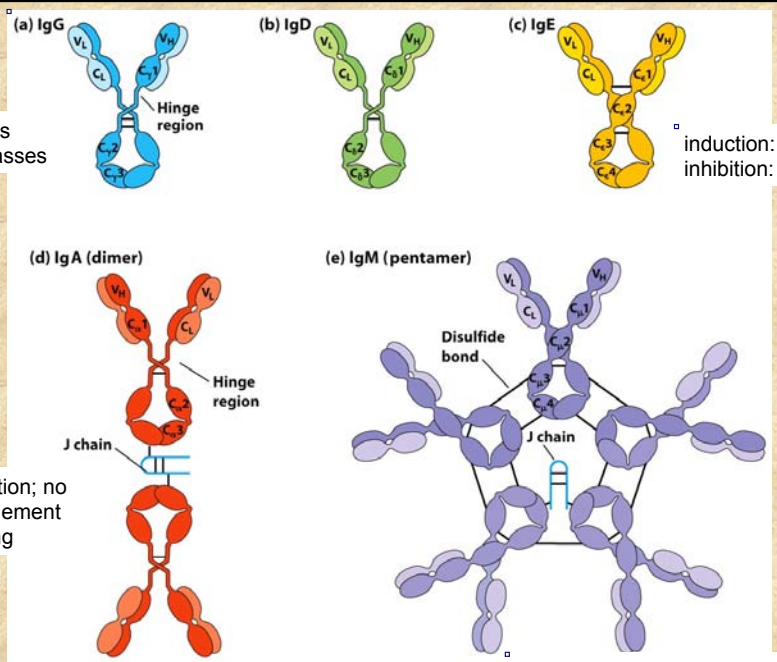
With each repetitive antigen exposure -
activation / "boost" :

- Germinal center of lymph follicles (white pulp spleen and lymph node)
- Point mutations in V_H V_L segments
- Some mutations result in higher affinity binding to antigen
- Increased activation of B cells with high affinity BCR / plasma cells with high affinity Ig
- Class switching

→ Affinity maturation

various subclasses

induction: Il-4
inhibition: IFN- γ



secretion; no complement binding

- Effective complement binding
- Immune complexes

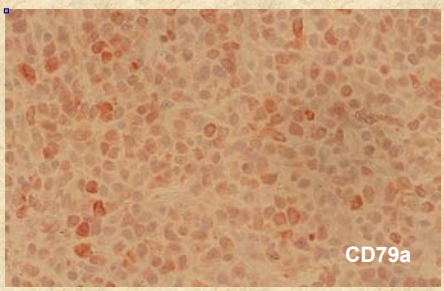
B cells / plasma cells in the skin

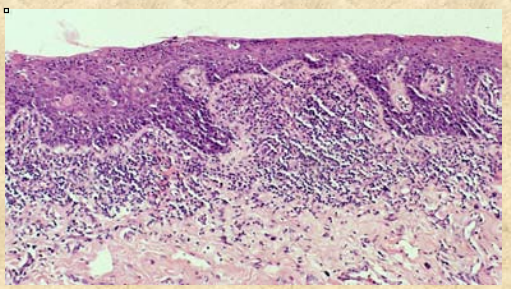
Normal haired skin:

- No B cells, no plasma cells

Recruitment upon inflammation

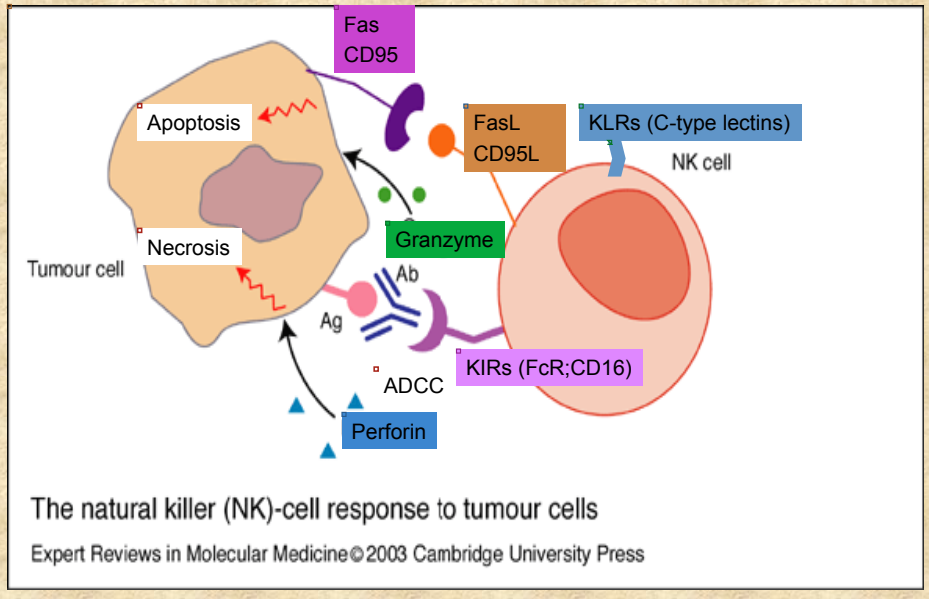
- Memory B cells
- Plasma cell rich: mucous membranes, paw-pads, m-c junctions





Natural Killer (NK-) cells

- Part of innate IS
- CD3ε



The natural killer (NK)-cell response to tumour cells

Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

Histiocytes

Progenitors CD34⁺

Macrophages

CD18/CD11b
CD18/CD11d (hematopoetic sites)

Receptors:

- FcR (CD16⁺)
- LPS R (CD14⁺)
- mannose-fucosyl R

MCH I
± MHC II

Myeloid DC

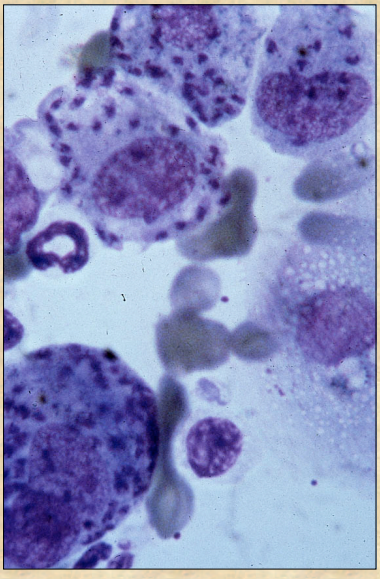
CD18/CD11c

Activated by:
IFN-γ, IL-3, GM-CSF
→ activated effector cells of AIS

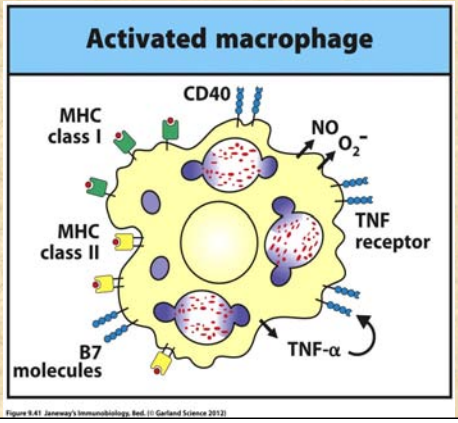
Phagocytosis

Activated macrophages are efficient effector cells:

killing of intracellular pathogens:
ex. Leishmania



oxygen free radicals
APC
TNF-α



Histiocytes

Progenitors
$CD34^+$

GM-CSF
TNF-α

Macrophages

Myeloid DC

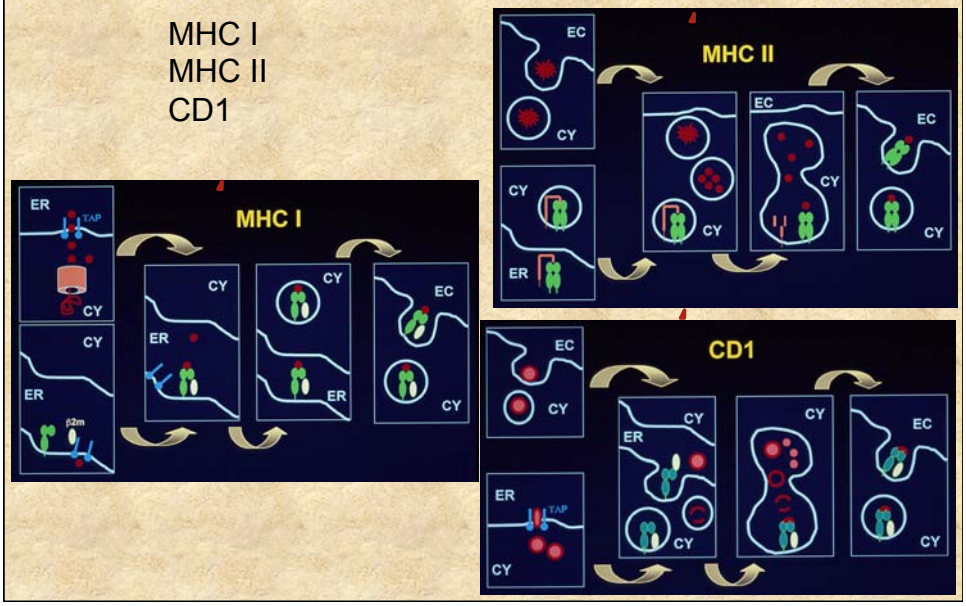
APC: antigen presentation
Subpopulations:
- Intraepithelial DC/ Langerhans cell
- Interstitial DC
- DC lymphoid organs (plasmacytoid DC)

PRR:
Membrane bound:
- TLR, R-kinase, CTR,

Intracytoplasmic:
- NLR, RLR,RNA helicases, NonRD kinases

DC - antigen presenting cells: dAPC

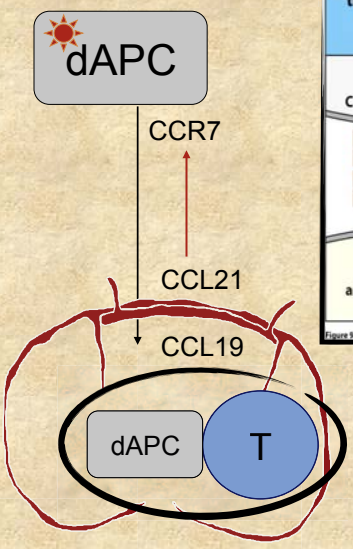
MHC I
MHC II
CD1

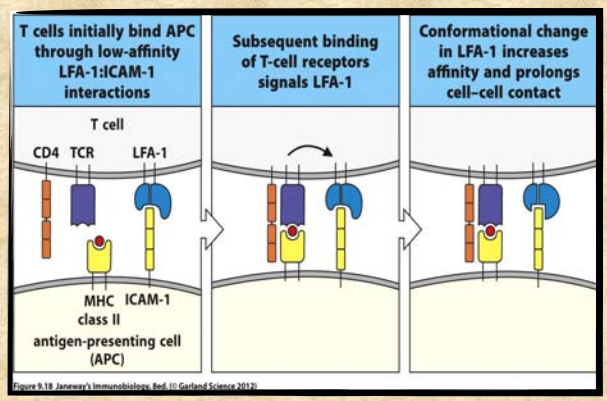


MHC complex genes - polymorphism

- MHC gene products are polymorphic → defines the range of antigens to which the individual immune system responds
- Control peptide recognition and binding by T cells ($CD8^+$ T cells, $CD4^+$ T cells)
- Non-self MHC are recognized by 1-10% of T cells

dAPC - adaptive immune response





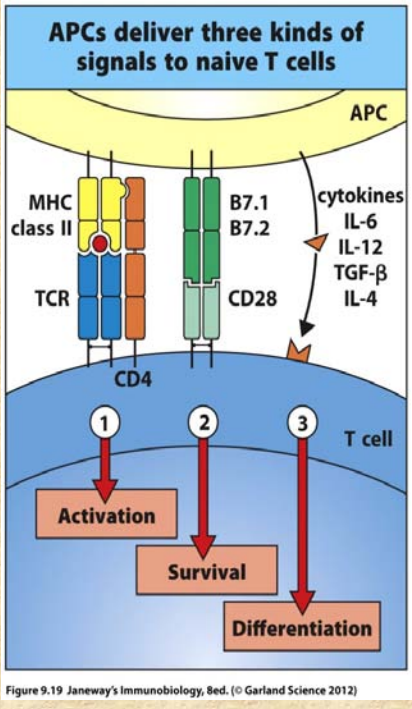
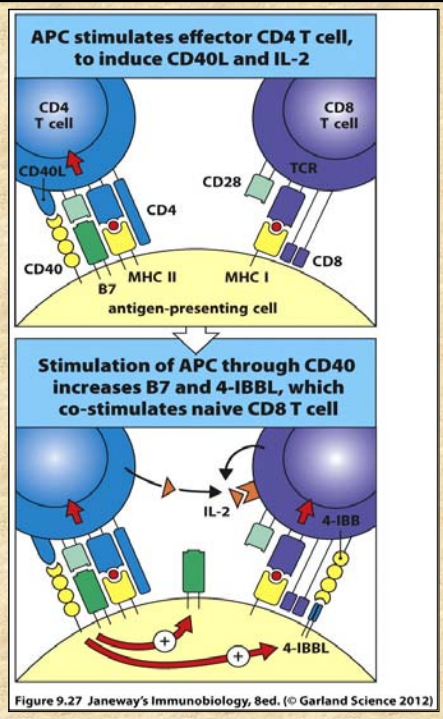
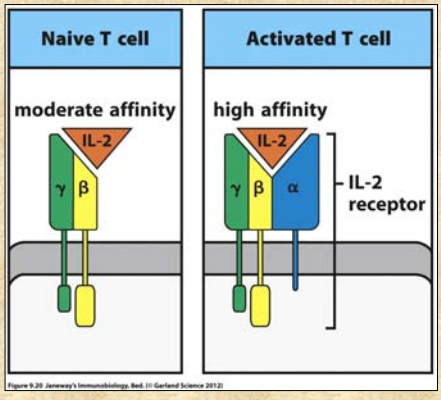


Figure 9.19 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

1. [illegible]
2. [illegible]
3. TREG
   TH17
   TH1 - helper cells
   TH2 - helper cells



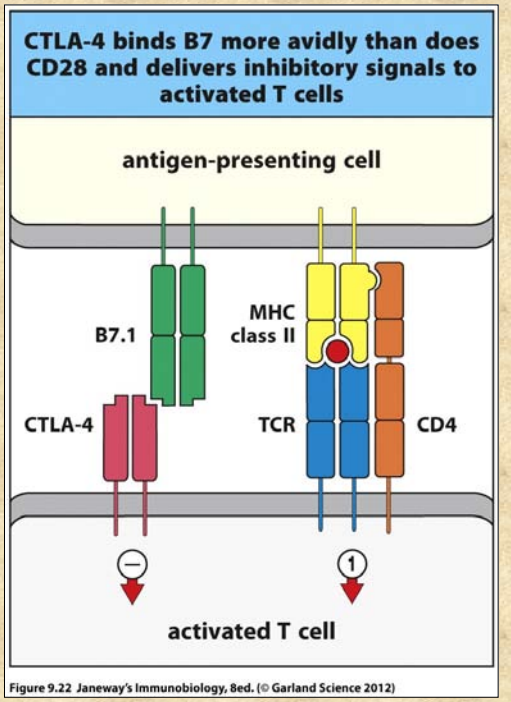


Down regulation of activating signal:

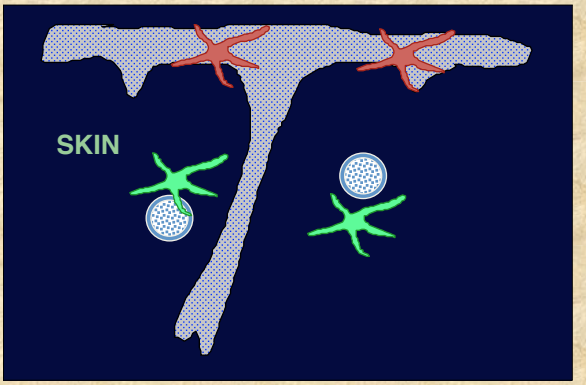
B7

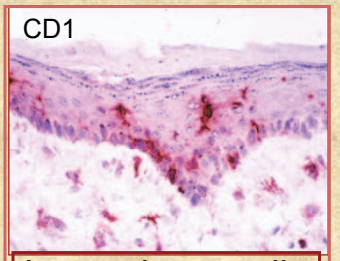
↕

CTLA-4



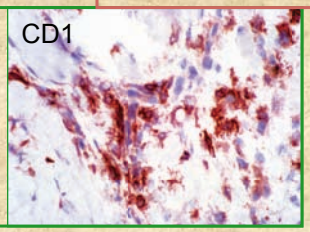
dAPC of the skin





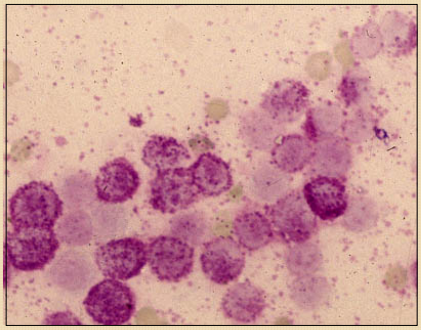
Langerhans cells
- E-cadherin^{+}
- Slow turnover
- Local self-renewal
- Rel. toleraogenic

dermal DC
- CD90^{+} (Thy-1)
- Fast turnover
- Renewal via blood born precursors
- Rel. immunogenic

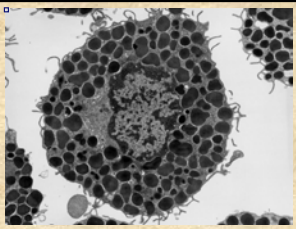


Mast cells

- SCF, c-kit, IL-5
- Differentiation in tissue
- Mouse: MMC and CTM
- Other species:
  tryptase
  chymase



Skin →
- connective tissue mast cells (CTM)
- perivascular dermis
- human: 7000/mm^3
- rarely in subcutis and epidermis
- dogs: more numerous on pinna and chin
- cat: more numerous on pinna and feet



Immediate response: degranulation

Ex: histamin, serotonin, TNF-α. ECF-A, NCF, kinonigenase

Late response: synthesized factors

Ex: LT, PAF, IL-3, IL-4, IL-5, IFN-γ, tryptase, chymase

- Type I hypersensitivity (IgE-FcεR1)
- Vascular tone, permeability
- Regulates inflammation: TREG, DC
- Activation of MBP, ECP released from eosinophils
- Mediates triggers from NS (substance P, neuropeptides)
- Tissue repair (TGF-β, basic-FGF, proteases)
- Angiogenesis (VEGF, PDGF, heparin)

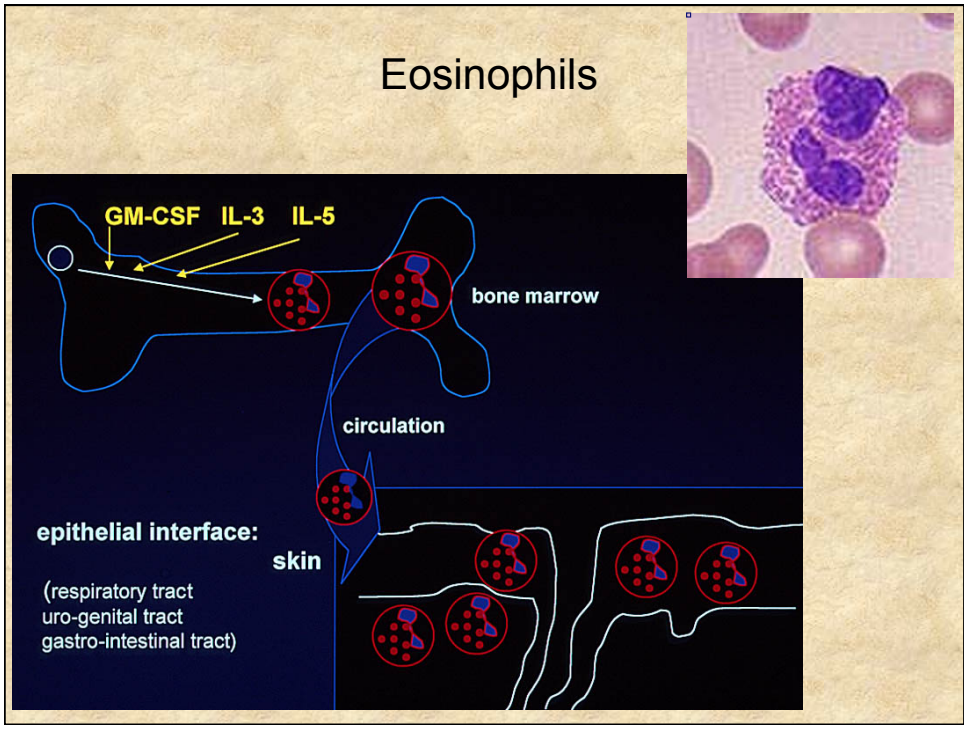
Basophils - recruitment to skin

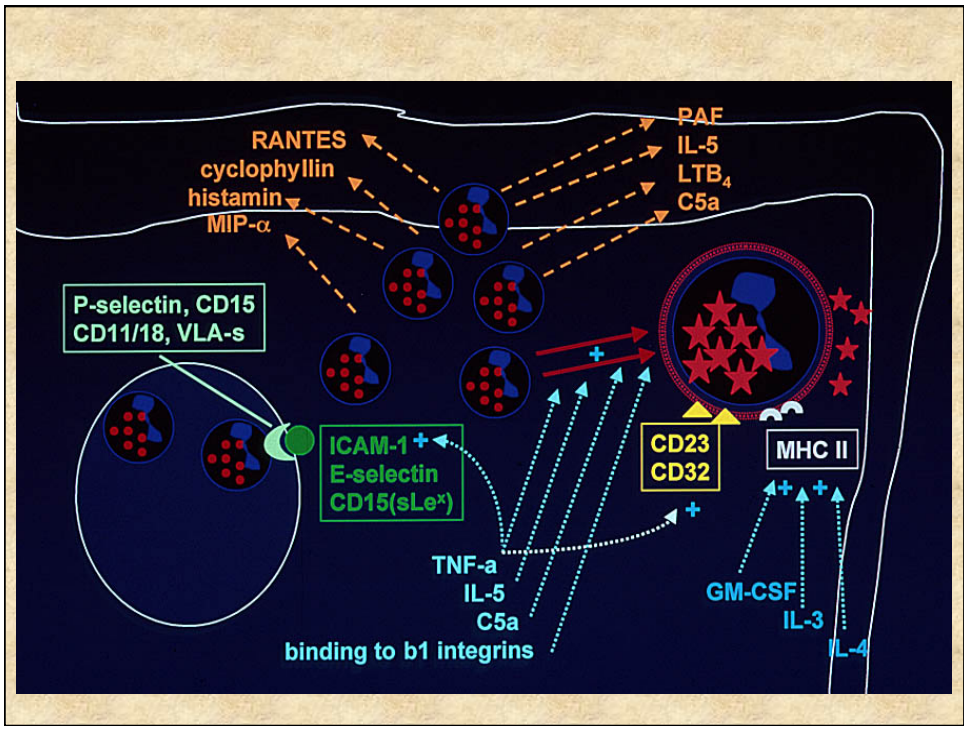
- IgE dependent reactions
- basophil hypersensitivity (DTH)
- tick manifestations in cattle
- produce IL-5:
  - ✓ chemotactic for eosinophils

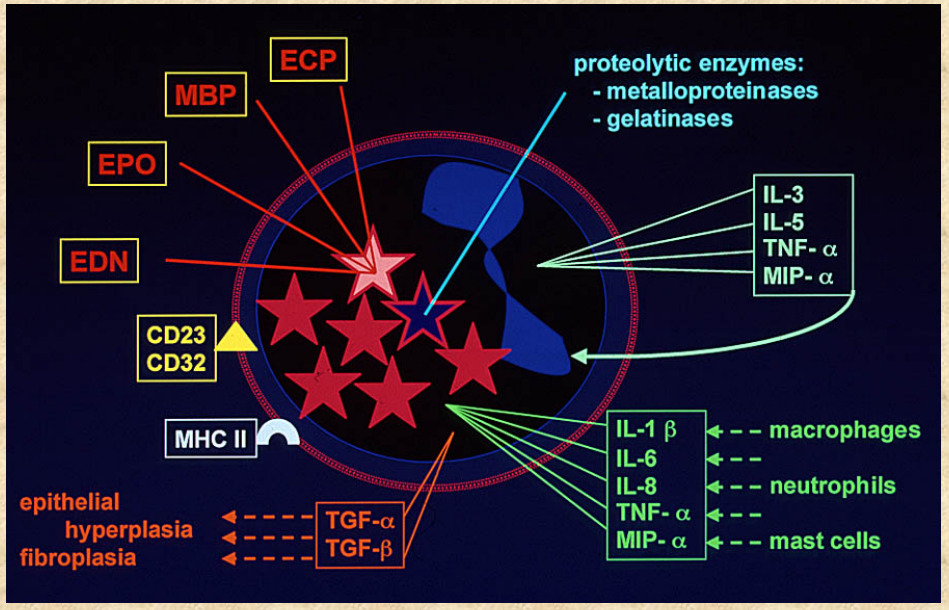
Neutrophils

Innate immune system

- Phagocytosis
- Proteolytic enzymes


Eosinophils
GM-CSF
IL-3
IL-5
bone marrow
circulation
epithelial interface:
skin
(respiratory tract
uro-genital tract
gastro-intestinal tract)


RANTES
cyclophyllin
histamin
MIP-α
PAF
IL-5
LTB4
C5a
P-selectin, CD15
CD11/18, VLA-s
ICAM-1
E-selectin
CD15(sLex)
CD23
CD32
MHC II
TNF-a
IL-5
C5a
binding to b1 integrins
GM-CSF
IL-3
IL-4

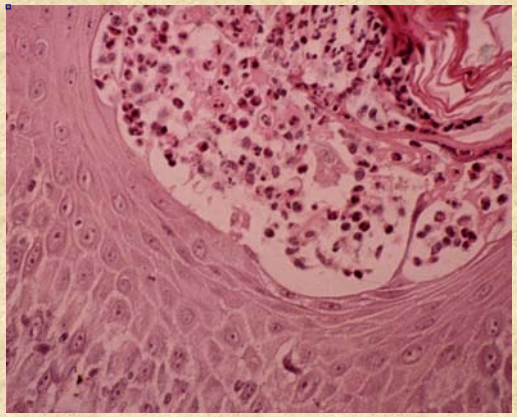


Eosinophils - skin

≠ Parasites

Type I hypersensitivity

Mast cell tumor



Examples:

- Furunculosis
- Pemphigus foliaceus
- Atopic dermatitis
- T cell lymphoma

SIS

SALT - skin associated lymphoid tissue

- keratinocytes
- lymphocytes
- Langerhans cells (LC)
- endothelial cells

Exo-SALT

✓ upper epidermis, γδ T cells

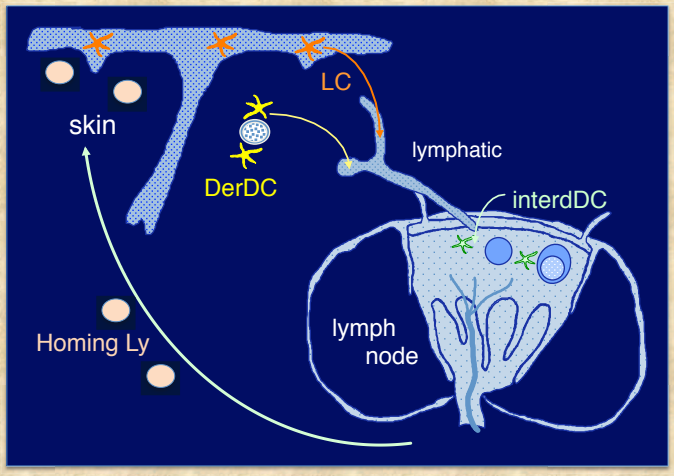
Endo-SALT

✓ lower epidermis, superficial dermis, αβ T cells

Skin Immune System

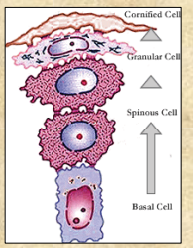
Innate IS

Adaptive IS



Induction of primary immune response
Immunity to previous encountered pathogens
Avoidance of deleterious immune responses (tolerance)

SIS - cellular components

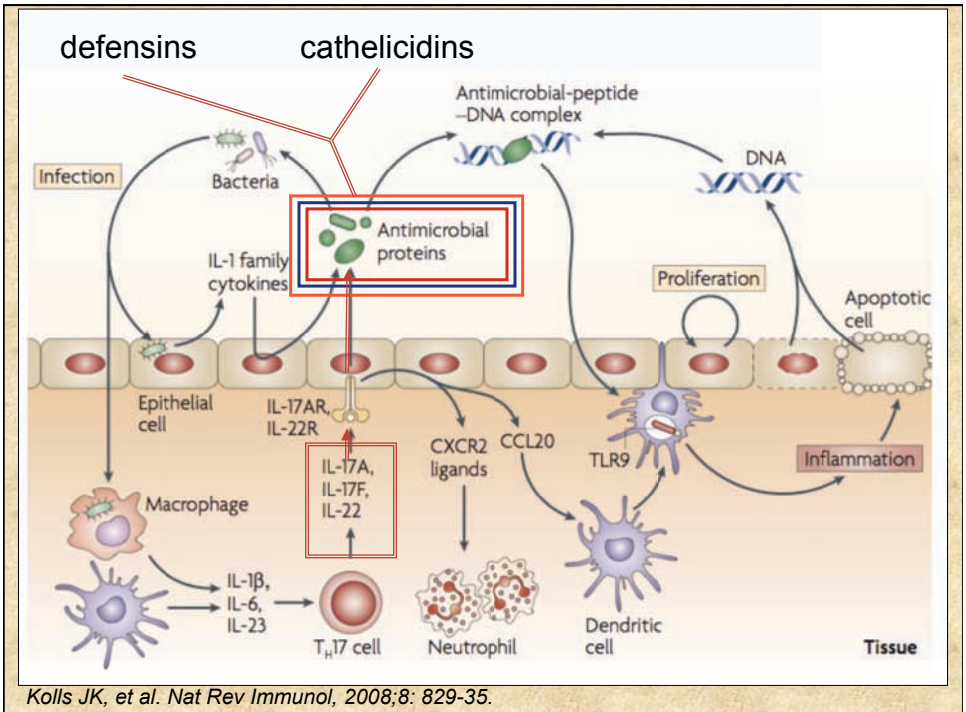


- Keratinocytes
- Dendritic APC:
  - epidermis, follicular epithelium: LC
  - dermis: Der DC
- Microvascular environment:
  - Endothelial cells PCV
  - Mast cells
- skin homing T cells:
  - αβ T cells
  - γδ T cells
- neural cells and neural dendritic processes
- draining lymph node and HEV

SIS - humoral components

Immediate response to PAMPs binding to PRR .

- Antimicrobial peptides
- Inflammasome
- Complement components
- Immunoglobulins
- Cytokines
- Chemokines
- Fibrinolysine
- Neuropeptides
- Eicosanoids
- Enzymes

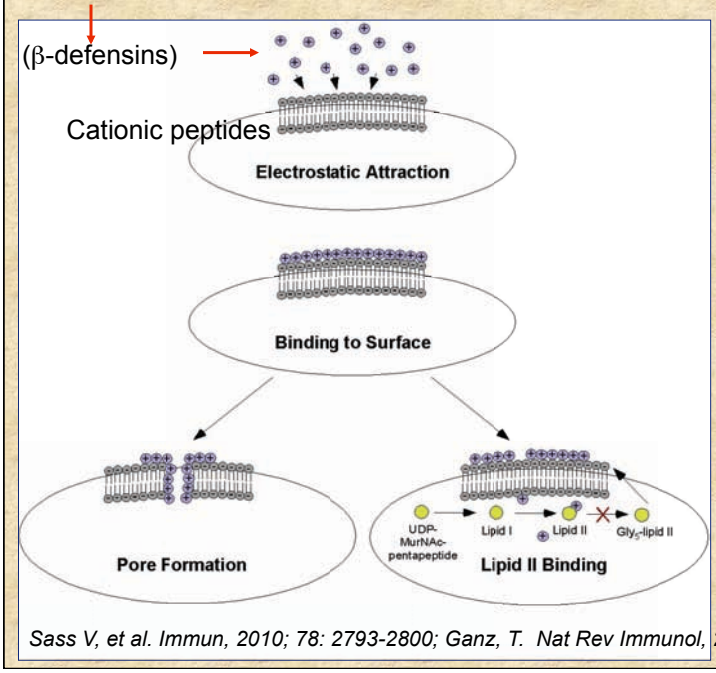


Kolls JK, et al. Nat Rev Immunol, 2008;8: 829-35.

Defensins

(β-defensins)

Cationic peptides



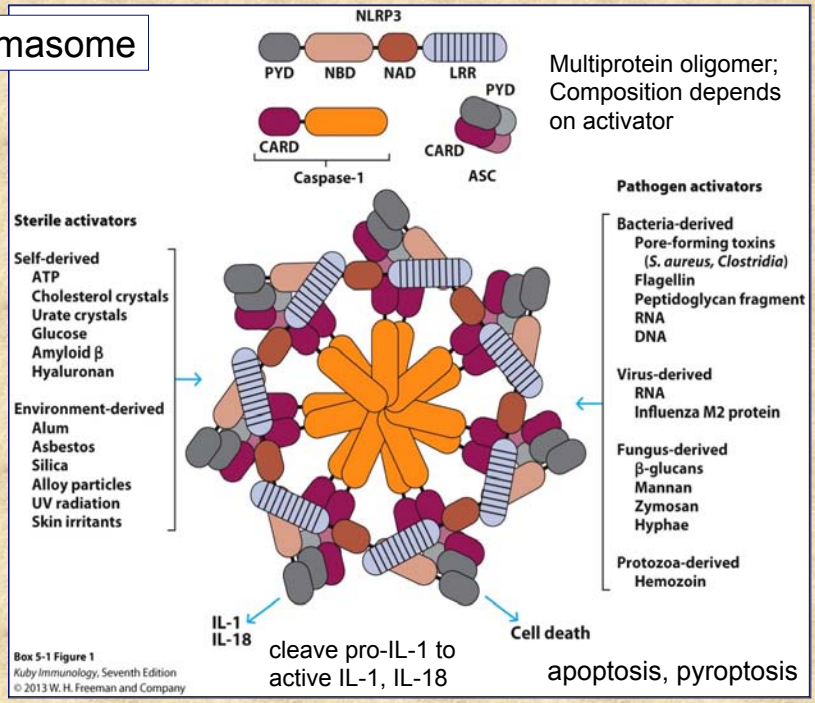
Cathelicidins

Pre-propeptide:

- signaling sequence
- propeptide
- mature peptide (C terminus)

Sass V, et al. Immun, 2010; 78: 2793-2800; Ganz, T. Nat Rev Immunol, 2003; 3: 710-20

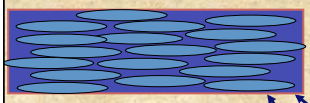
Inflammasome



cleave pro-IL-1 to active IL-1, IL-18

apoptosis, pyroptosis

The Barrier



Keratinocytes and envelope:
20% lipids

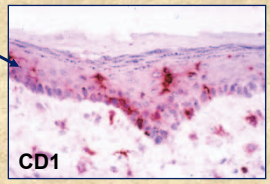
Sphingolipids
Cholesterol
Free fatty acids

- ✓ "protective lipids"
- ✓ iNOS
- ✓ Antimicrobial peptides

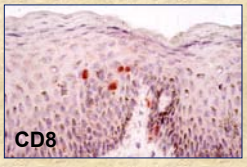
Microbiom

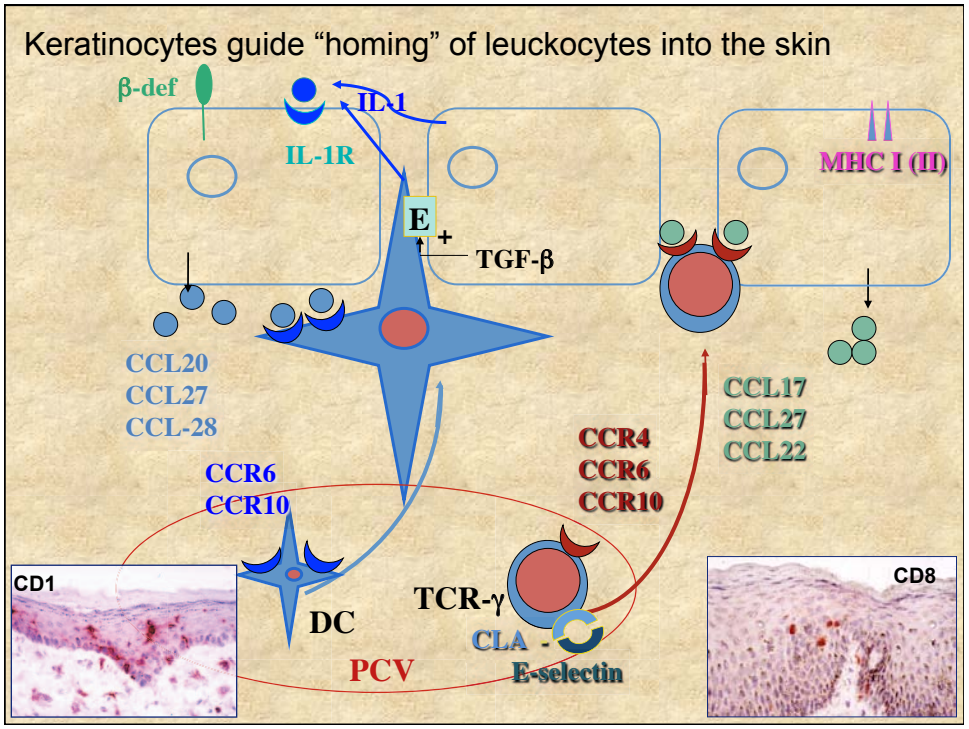
Foll KC

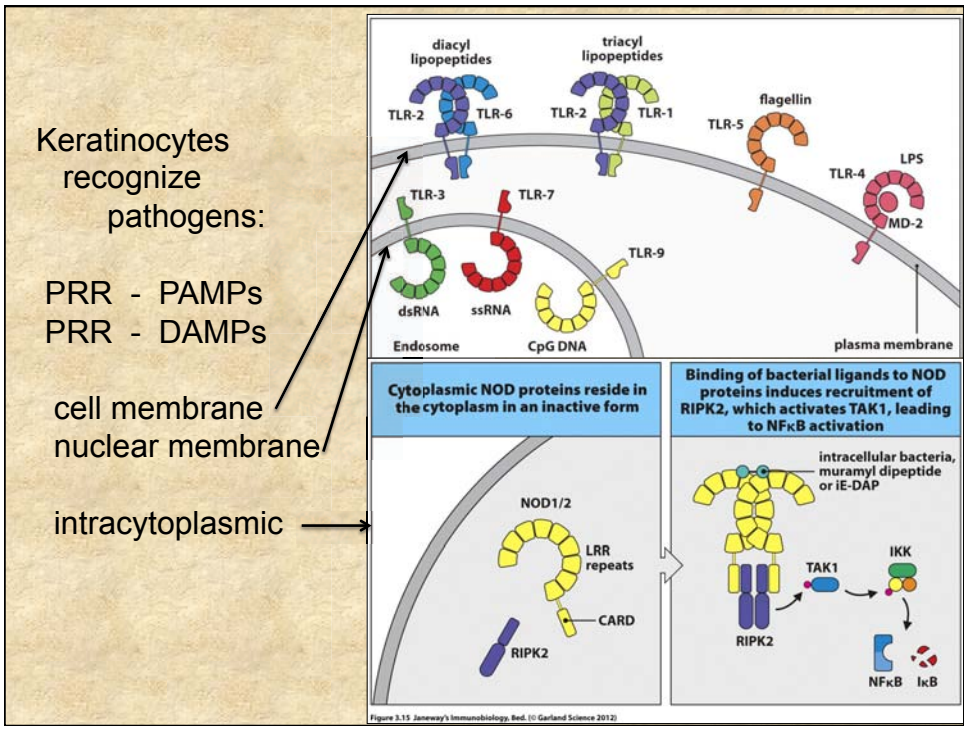
Langerhans cells



Lymphocytes



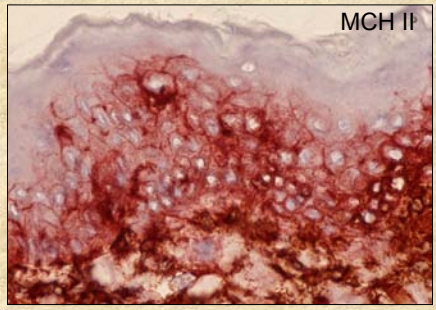

Keratinocytes guide “homing” of leuckocytes into the skin
β-def
IL-1
IL-1R
MHC I (II)
E
+
TGF-β
CCL20
CCL27
CCL-28
CCR6
CCR10
CCR4
CCR6
CCR10
CCL17
CCL27
CCL22
CD1
DC
TCR-γ
CLA
E-selectin
PCV
CD8


Keratinocytes recognize pathogens:
PRR - PAMPs
PRR - DAMPs
cell membrane
nuclear membrane
intracytoplasmic
diacyl lipopeptides
triacyl lipopeptides
TLR-2
TLR-6
TLR-2
TLR-1
flagellin
TLR-5
LPS
TLR-4
MD-2
TLR-3
TLR-7
TLR-9
dsRNA
ssRNA
CpG DNA
Endosome
plasma membrane
Cytoplasmic NOD proteins reside in the cytoplasm in an inactive form
Binding of bacterial ligands to NOD proteins induces recruitment of RIPK2, which activates TAK1, leading to NFκB activation
NOD1/2
LRR repeats
CARD
RIPK2
intracellular bacteria, muramyl dipeptide or iE-DAP
TAK1
IKK
RIPK2
NFκB
IκB

Keratinocytes are APC

- MHC I → TCR – CD8+
- MHC II → TCR – CD4+

- B7 → CD28
- CD40 → CD40L
- ICAM-1 → B2 integrins



"Immune privilege" of anagen hair follicle: MHC I low/neg

Immune-suppressive factors:
α-MSH
IGF-1
IL-10
TGF-β

↓

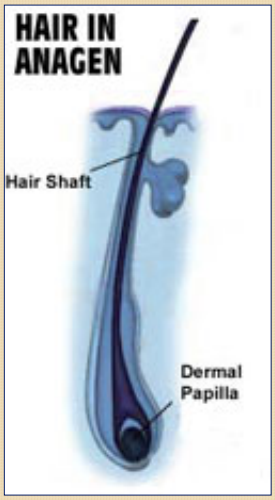
Counteract IFN-γ

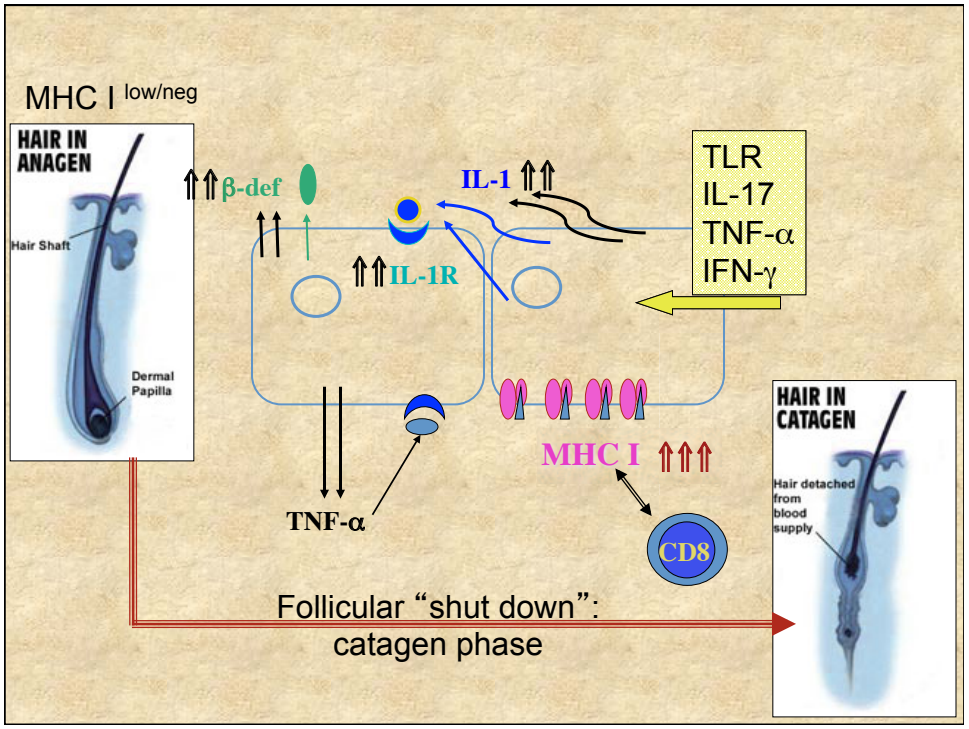
↓

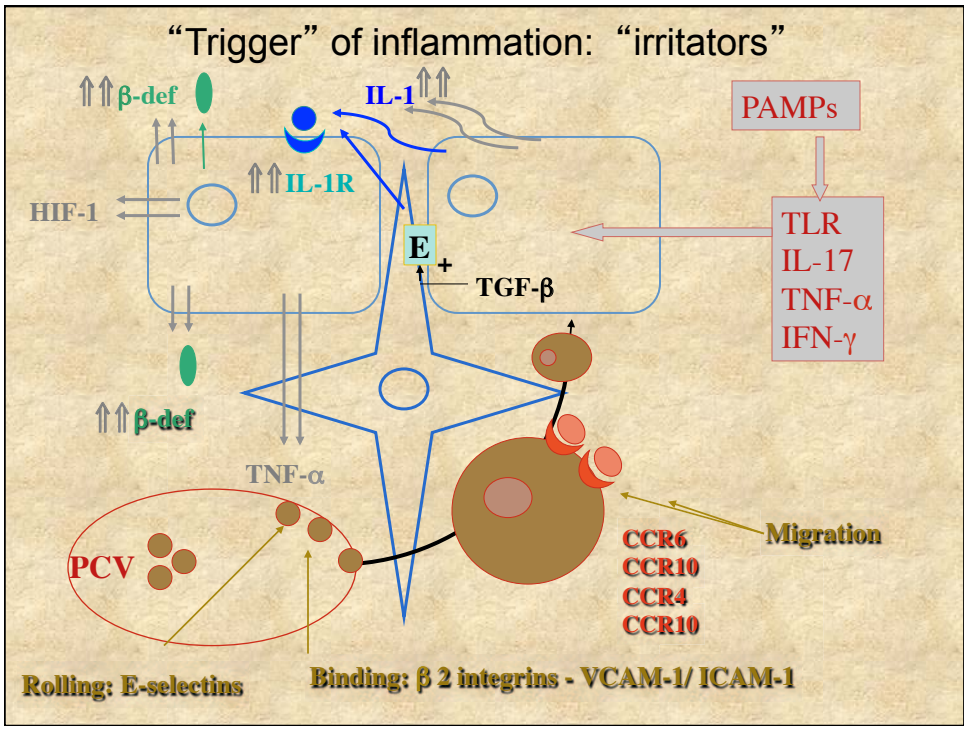
Sequester follicular auto-antigens (anagen/ melanogenesis)

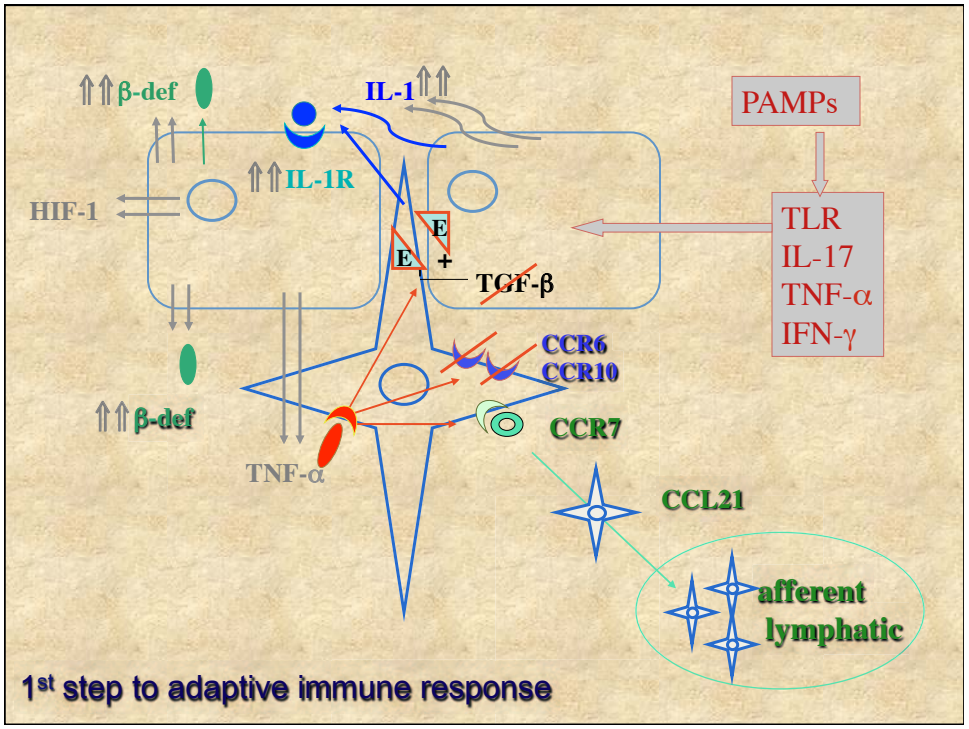
↓

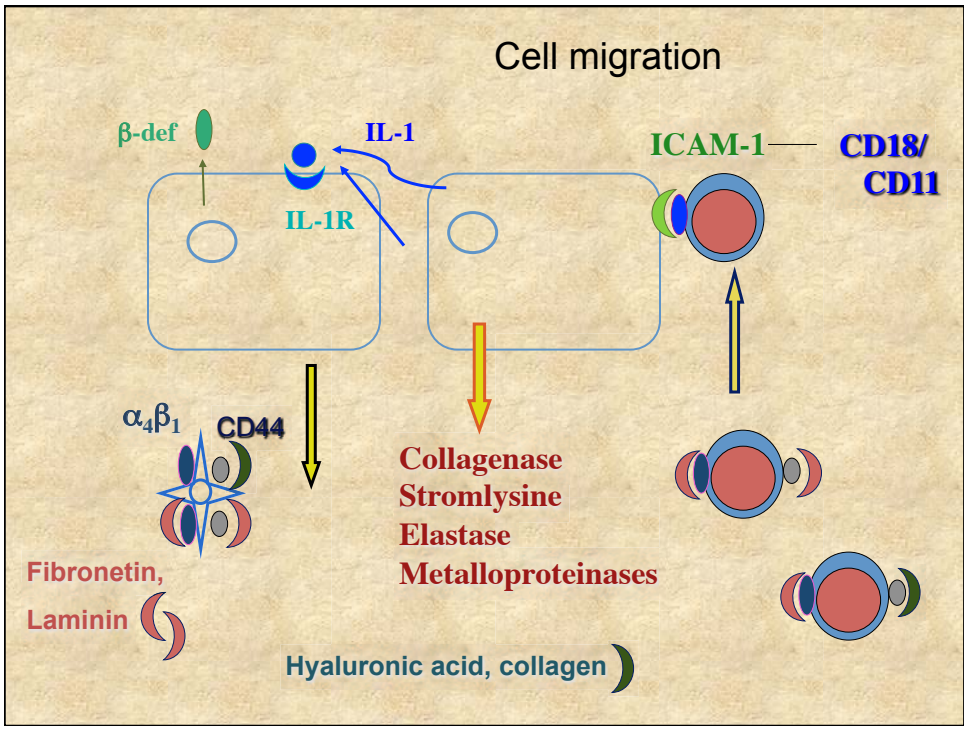
Not accessible to $CD8^+$ T cells

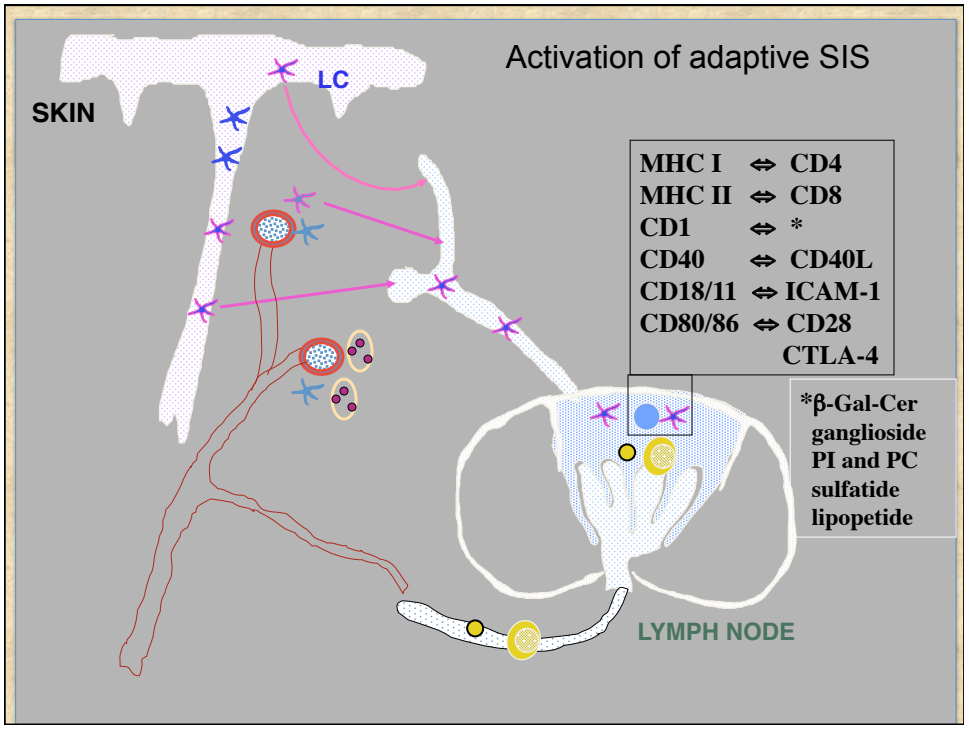


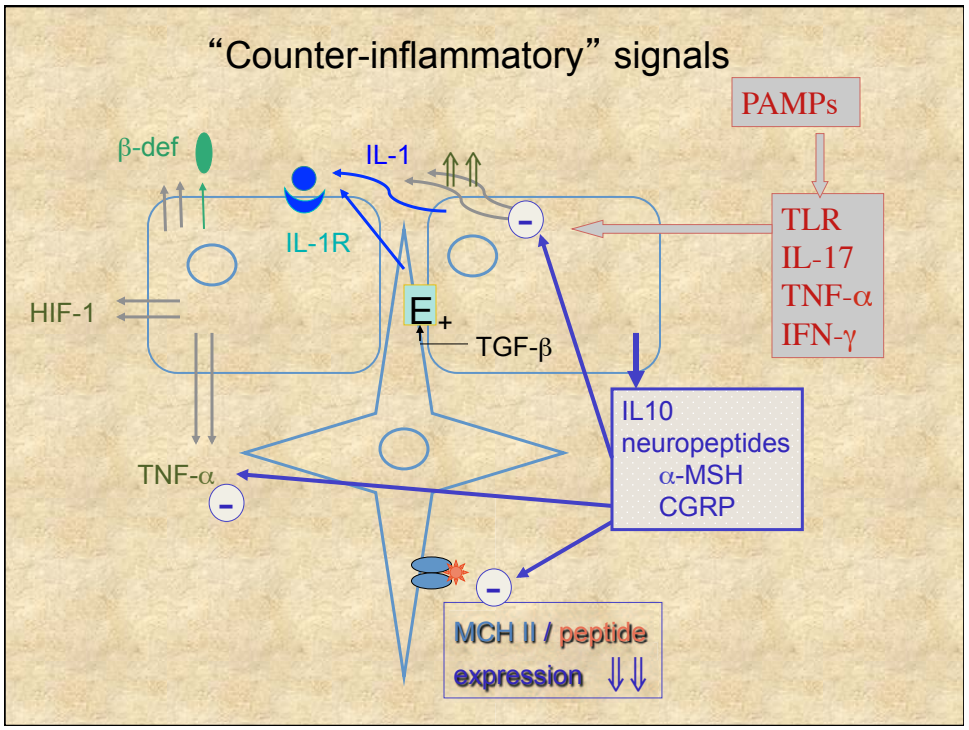

MHC I low/neg
HAIR IN ANAGEN
Hair Shaft
Dermal Papilla
β-def
IL-1
IL-1R
TLR
IL-17
TNF-α
IFN-γ
MHC I
TNF-α
CD8
HAIR IN CATAGEN
Hair detached from blood supply
Follicular "shut down": catagen phase

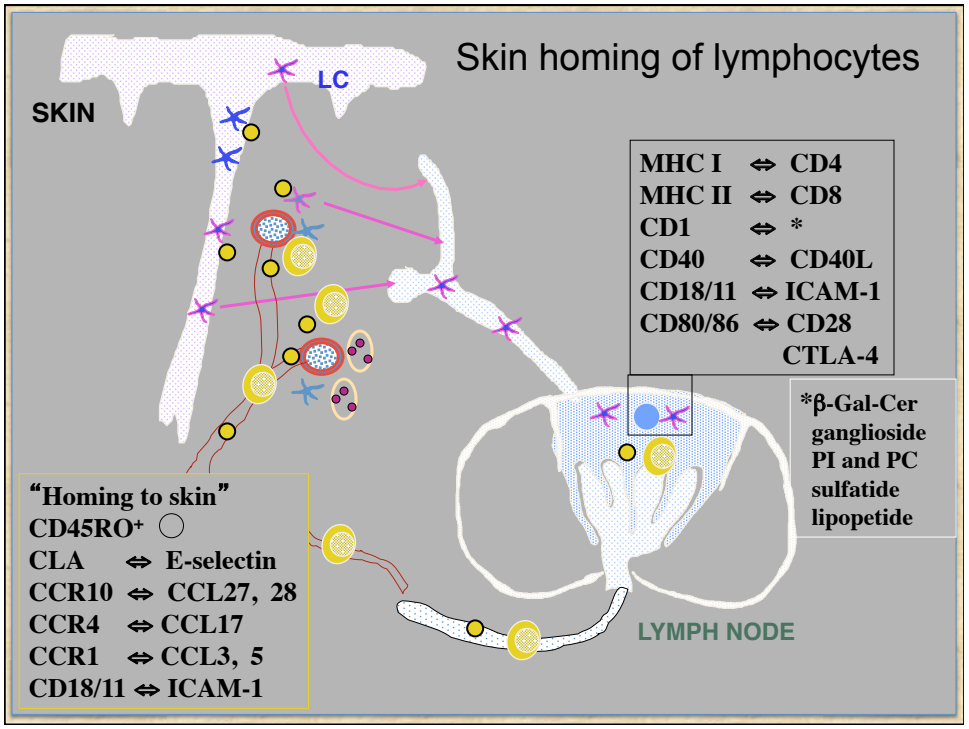

"Trigger" of inflammation: "irritators"
β-def
IL-1
IL-1R
PAMPs
HIF-1
E
TGF-β
TLR
IL-17
TNF-α
IFN-γ
β-def
TNF-α
PCV
CCR6
CCR10
CCR4
CCR10
Migration
Rolling: E-selectins
Binding: β 2 integrins - VCAM-1/ ICAM-1


β-def
IL-1
PAMPs
IL-1R
HIF-1
TLR
IL-17
TNF-α
IFN-γ
E
E
+
TGF-β
CCR6
CCR10
β-def
CCR7
TNF-α
CCL21
afferent
lymphatic
1st step to adaptive immune response


Cell migration
β-def
IL-1
ICAM-1
CD18/
CD11
IL-1R
α4β1
CD44
Collagenase
Stromlysine
Elastase
Metalloproteinases
Fibronetin,
Laminin
Hyaluronic acid, collagen


Activation of adaptive SIS
SKIN
LC
MHC I ⇔ CD4
MHC II ⇔ CD8
CD1 ⇔ *
CD40 ⇔ CD40L
CD18/11 ⇔ ICAM-1
CD80/86 ⇔ CD28
CTLA-4
*β-Gal-Cer
ganglioside
PI and PC
sulfatide
lipopetide
LYMPH NODE


"Counter-inflammatory" signals
PAMPs
β-def
IL-1
IL-1R
HIF-1
E
TGF-β
TLR
IL-17
TNF-α
IFN-γ
IL10
neuropeptides
α-MSH
CGRP
TNF-α
MCH II / peptide
expression ⇓⇓



skin homing T cells:
Memory cells

skin homing B cells:
Memory cells

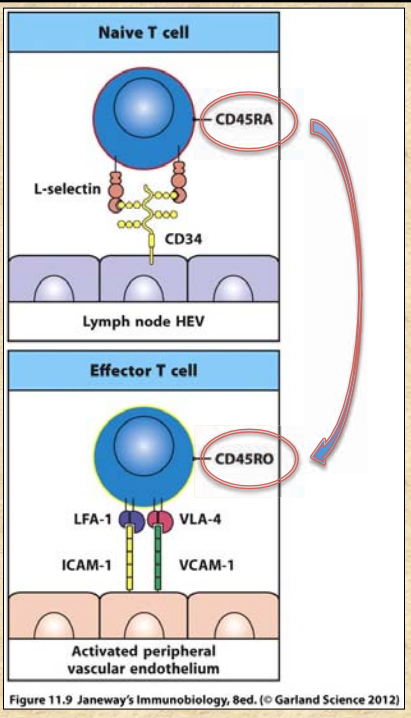
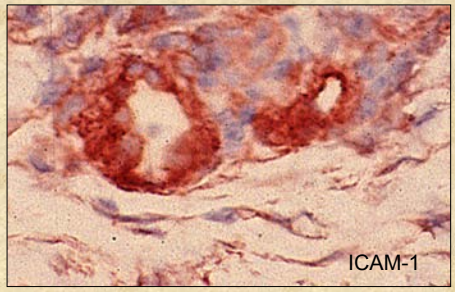


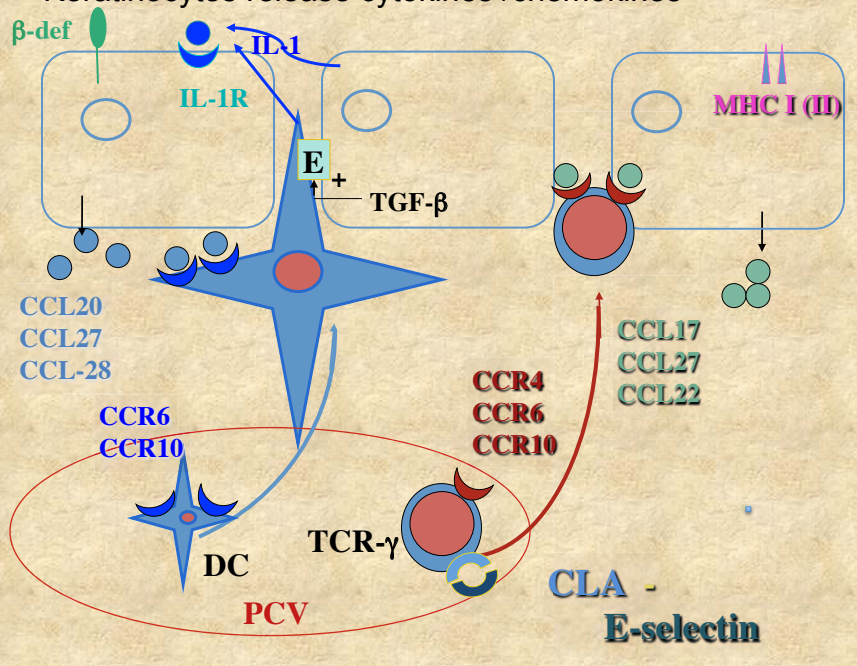
Figure 11.9 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

SIS – microvascular environment

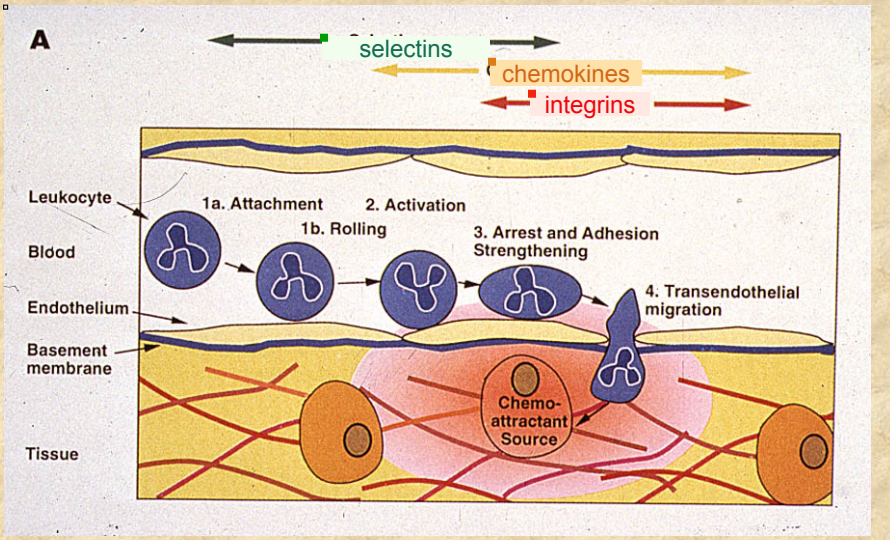


normal skin	
ICAM-1 (CD54) ICAM-2 (CD50)	β2 integrins on leukocytes
inflamed skin	
ICAM-1 upregulated	β2 integrin ligation
E-selectin expresses ($CD62_E$)	CLA antigen
VCAM-1 expressed (CD106)	β1 integrins: VLA-4 / (CD49d/CD29)

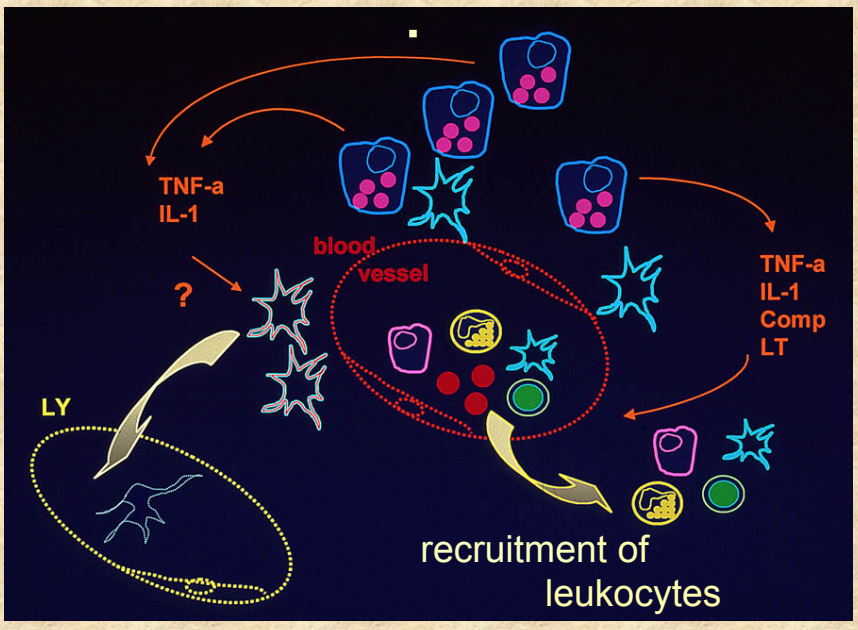
Keratinocytes release cytokines /chemokines



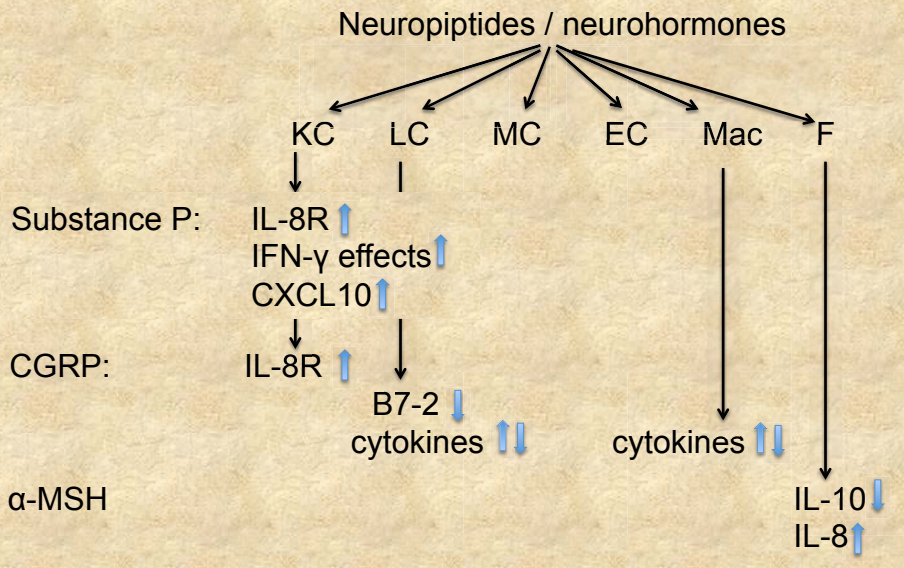
Leukocyte migration - inflammation



Cutaneous mast cells – microvascular unit



SIS - neural/peptides



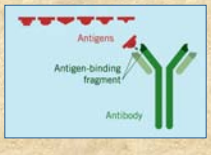
Anergy / tolerance

- Antigen overload: T cell apoptosis
- Absence of costimulation (B7, CD28, CD40, CD40L)
- TREG: IL10, TGF-β; suppression of TH response
- Lack of γδ-T cells: impared tolerance
- Antigen-specific suppression

Autoimmune disease

- Lack of central tolerance: AIRE – mutation
- Lack of peripheral tolerance: T_{REG} insufficiency
- Exposure to cryptic antigens (ex: nuclear antigens)
- Alteration of self by haptens (ex: drugs)
- Cross-reactivity: low affinity B cell receptor may react with similar antigen
- Antigen spreading: T cell activated by a new antigen may subsequently recognize similar self antigen
- Genetic make-up: MHC
- Contribution of T_{H17}: amplify immune response
- IL-23: binds to p40 subunit of IL-12 receptor

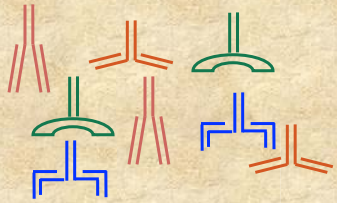
Techniques



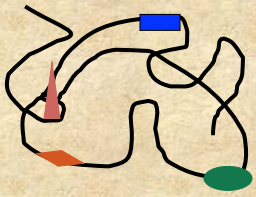
Immunohistocheistry

Monoclonal Ab
recognizes a *single* epitope

Polyclonal Ab
recognizes *multiple* epitopes



Monoclonal Ab
high specificity
± sensitivity



Polyclonal AB
± specificity
higher sensitivity

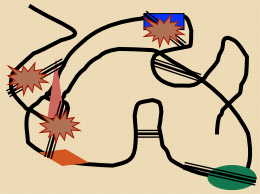
"know your antibody"

Know what to expect!!!

Use correct controls!

Species specificity
Species it has been produced in

Does antibody work in formalin-fixed tissue?



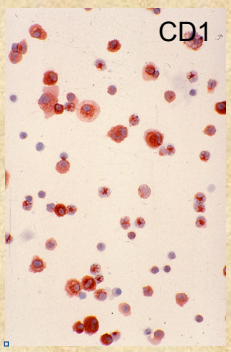
Antigen retrieval:
- enzymatic
- citric acid
- heat

Imprints / aspirates / snap frozen tissue

- avoid chrystals/clefts

Air- dried:

- at +4°C (1-2 days)
- keep frozen -20°C (with dessicator)



- small piece in cryomold
- add OCT (liquid at room temperature)
- remove all air bubbles

- methylbutane in small flask
- liquid nitrogen in cryocontainer
- immerse flask with methylbutane in container with liquid nitrogen

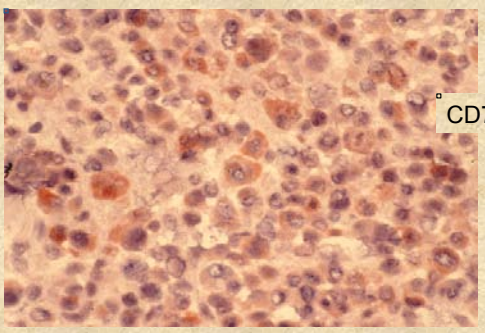
- bottom and and rim solid - center liquid
- carefully immerse cryomold into methylbutane until solid frozen (20-40 seconds)

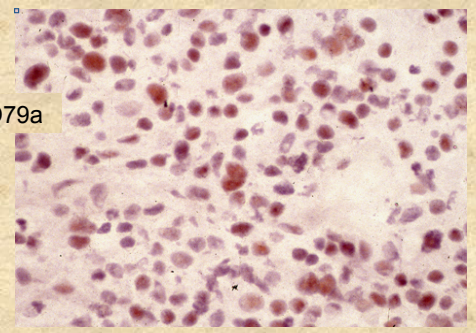
- keep block at -70°C in plastic wrap

- keep frozen at any times!!
- sections cut with cryotome

"know your antibody"

- Know the location of the protein of interest!





"Negative result"

- make sure you compare the controls
- with tumors - may not rule completely rule out a cell type (tumor cells change expression of proteins)

Cross-reactivity of antibodies

1. Does the Ab work in a different species? (best for highly conserved antigens)
2. Is the Ab recognizing the correct antigen in a different species? (species-specific differences of expression, ex: CD4, CD80, CD86; background issues)

+ control: tissues from primary species

identification of the protein

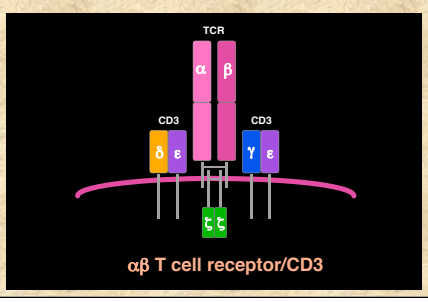
- Immunohistochemistry / immunofluorescence
- Flow cytometry
- Immunoprecipitations / immunoblot
- Amino acid sequencing of the antigen

Clonality testing

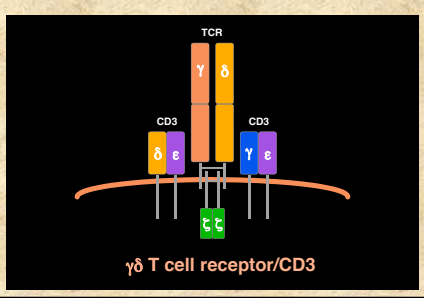
Identification of polyclonal vs. clonal T and/or B cell populations
reactive vs. neoplastic

T cells

- T cells rearrange their T cell receptor TCR (RAG-1, RAG-2 genes)
  - TCR αβ
  - TCR γδ
- each T cell has a unique sequence and length of the TCR



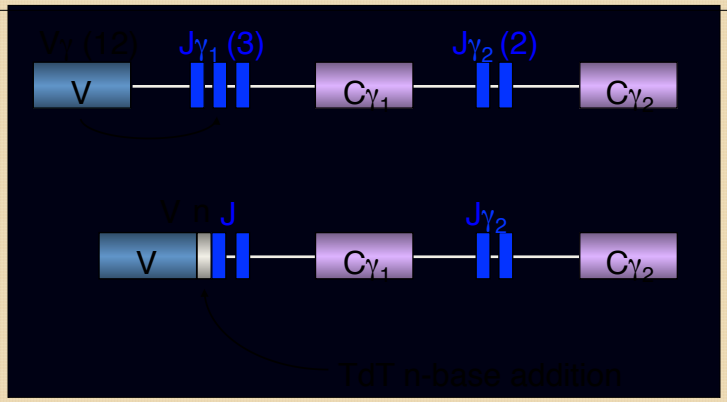
αβ T cell receptor/CD3

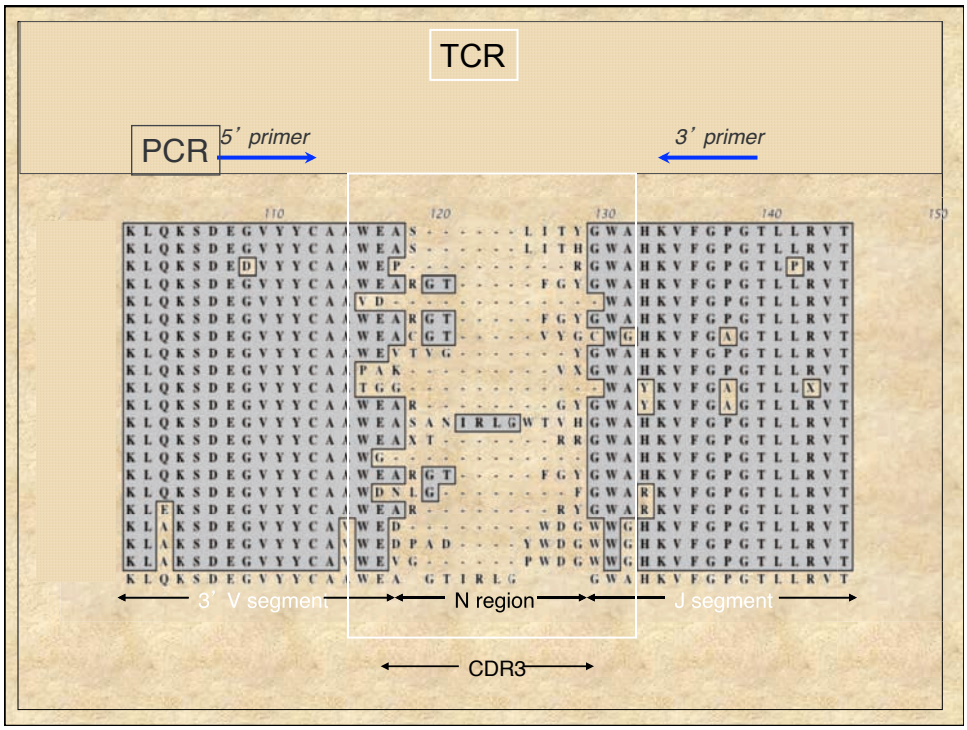


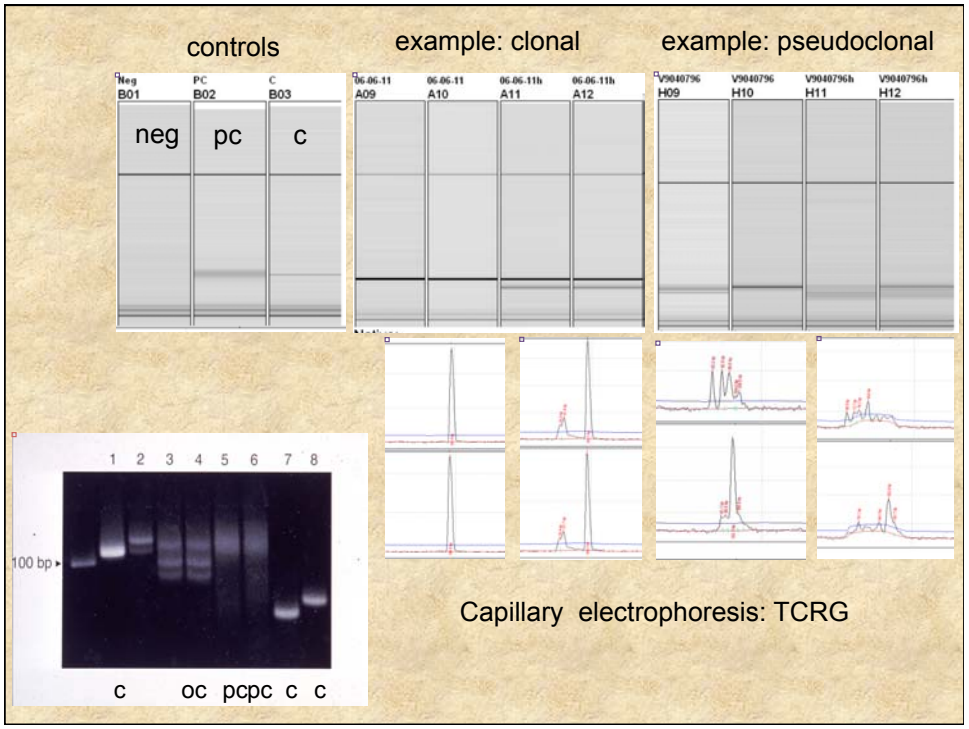
γδ T cell receptor/CD3

TCR gene rearrangement - TCRG preferred target for clonality testing

- most TCRαβ rearrange TCRG prior to TCRB
  γδ-T cells and αβ-T cells rearrange TCRG
- structure of TCRG less complex than TCRB
  high homology (lack of D segment)







Clonality testing

- B cells

B cells rearrange their B cell receptor IgH -

- variable region of the released antibody

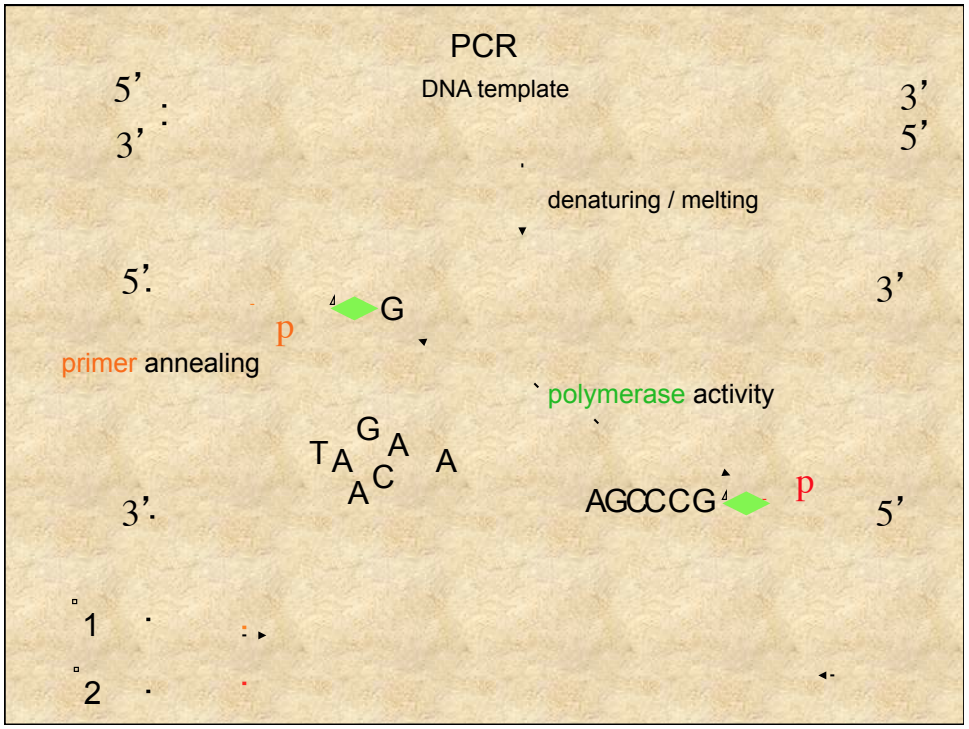
Each B cell has a unique sequence and length of the IgH variable region

KDE: κ- deleting element: λ or κ light chains: V and J region

- repetitive exposure to antigen

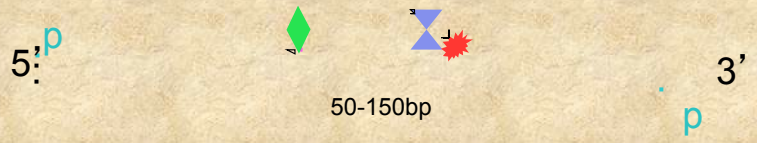
Ab class "switching"

Additional mutations:
Primers may no longer be able to bind – fasle polyclonal response

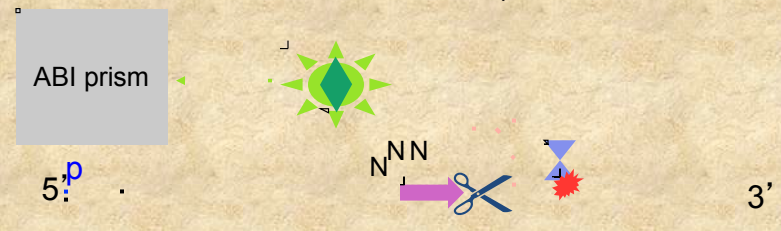


Quantitative PCR - TaqMan

3 probes: Primers (p), internal TaqMan probe (ITMP), reporter dye (FAM), quentcher dye (TAMRA), phosphate blocker (P)



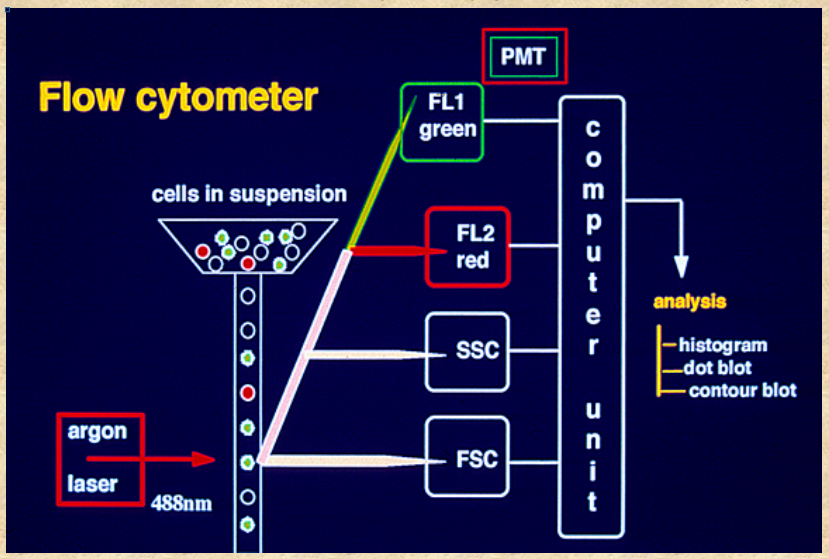
- DNA polymerase has 5'-3' exonuclease activity
- Release of fluorescent light measured every 7 sec
- Intensity of released fluorescent dye directly related to amount of cDNA in the sample



Flow cytometry

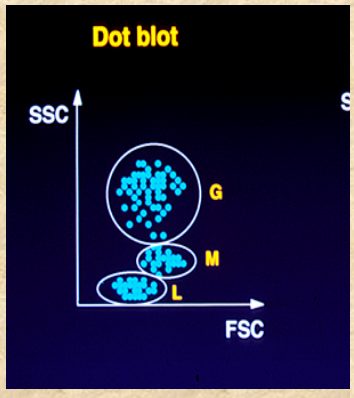
Quantitative analysis of cells in solution

Evaluation of individual cells labeled by Ab conjugated with fluorescent dye



Flow cytometry - peripheral blood

(Citrate, EDTA)



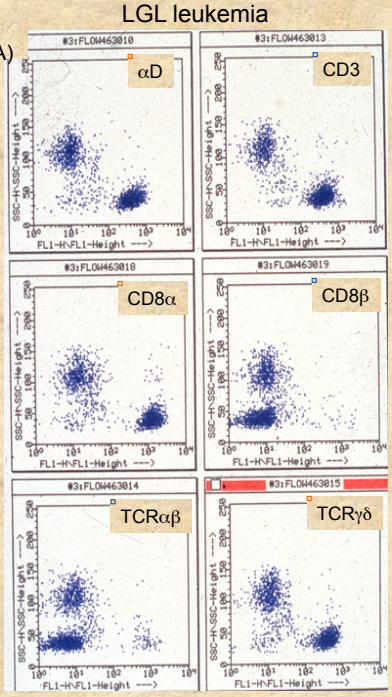
Diagnostic purposes

- ✓ immunophenotyping of cells in solution (aspirates, cell suspensions)
- ✓ peripheral blood: leukemia

Clinical follow-up

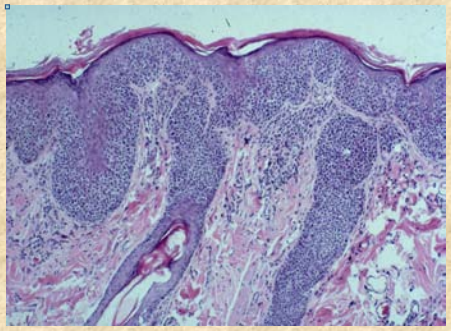
- ✓ residual disease / success of therapy

LGL leukemia



Example: Airdale terrier, 10 year old, fs

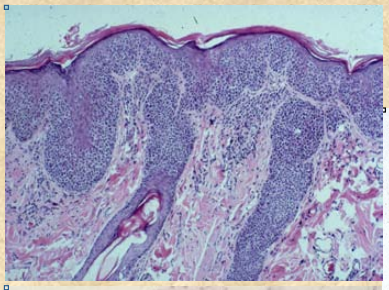
- generalized skin ulcers
- worse on caudal dorsum and perineum

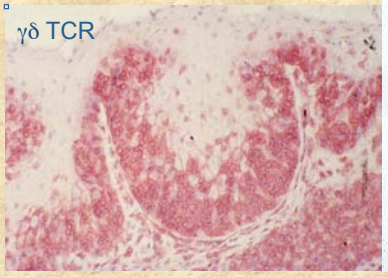


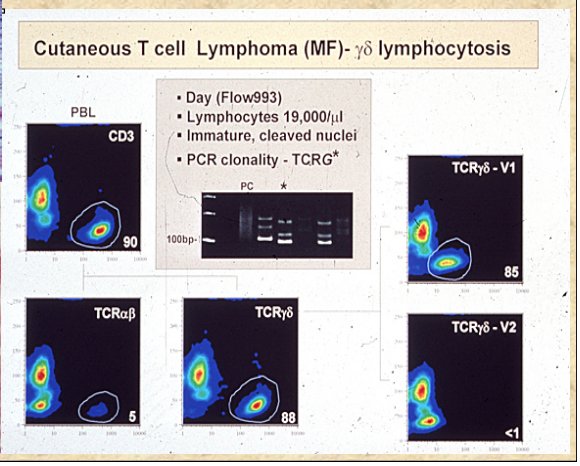
epitheliotropic lymphoma

- Skin ulcers
- HCT=39.8%
- platelets = 523,000/ul
- WBC = 43,300/ul
- neutrophils = 19.052/ul
- lymphocytes = 19,052/ul
- serum calcium = 12.2md/dl

Airdale terrier, 10 year old, fs

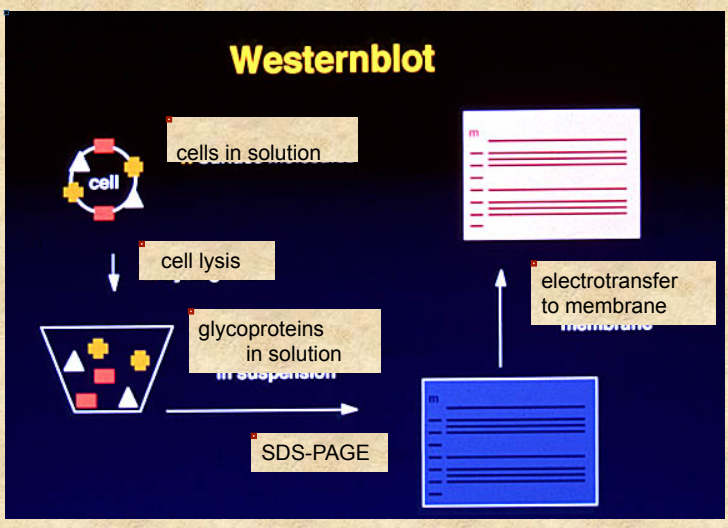




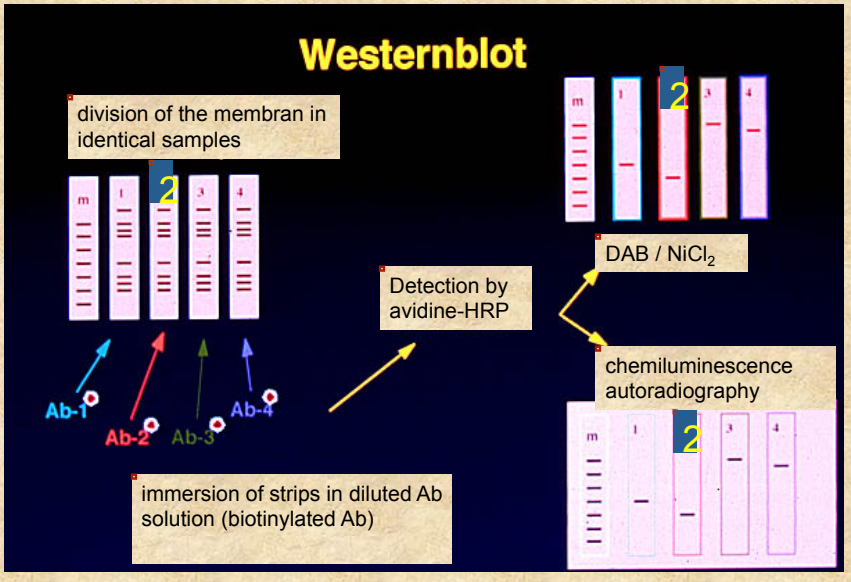


Western blot

Identification of a (glyco-)protein based in its molecular weight: cytoplasmic and membrane-bound proteins

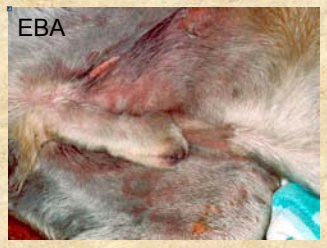


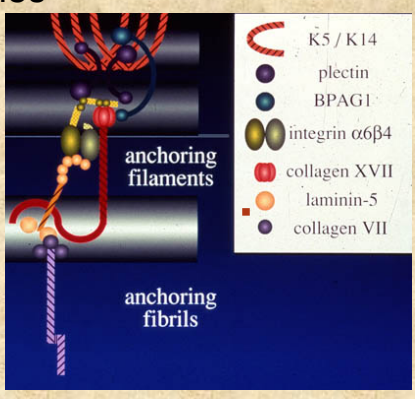
Western blot

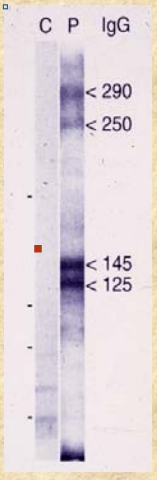


Western blot

- identification of antibodies in patients serum:
  - ✓autoantibodies

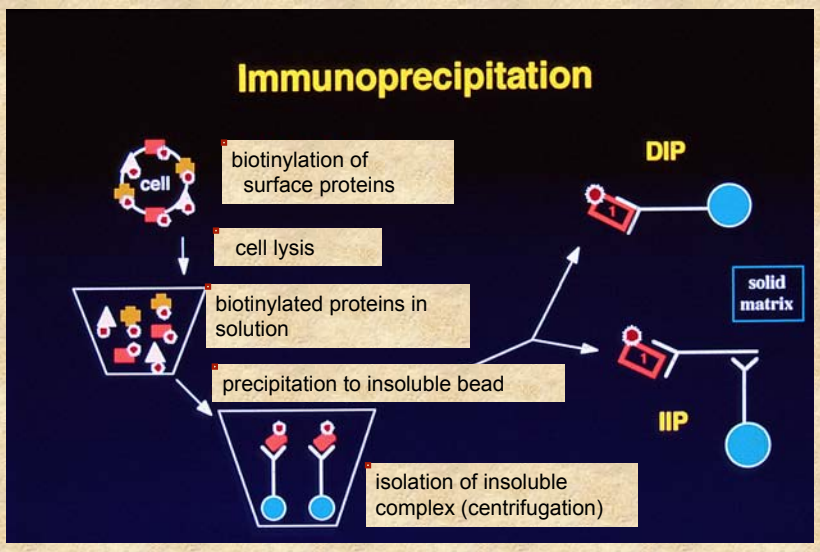






Immunoprecipitation - immunoblot

Identification of a (glyco-)protein based in its molecular weight: membrane-bound proteins



Immunoprecipitation - immunoblot