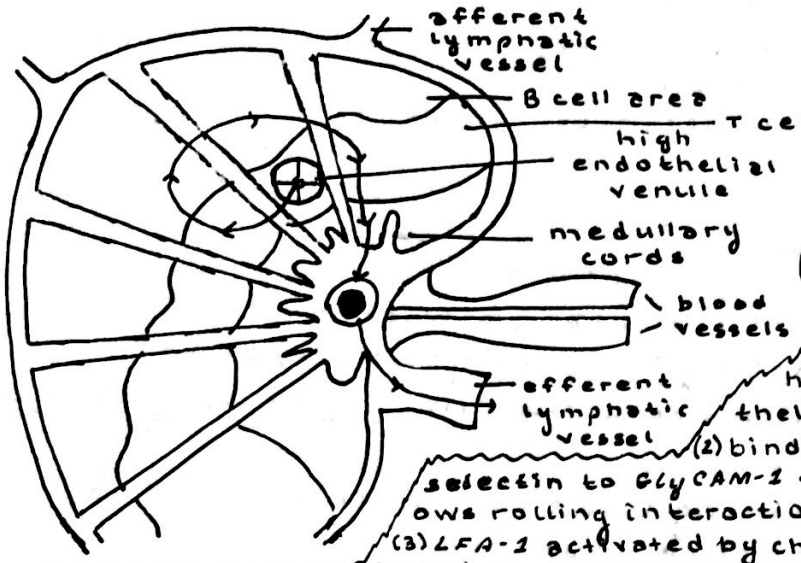
EXAM 3 STUDY GUIDE

- Lymph Node + System Architecture



* T cell entry into lymph nodes
(1) enters high endothelial venule
(2) binding of L-selectin to GlyCAM-1 + CD34 allows rolling interaction
(3) LFA-1 activated by chemokines bound to extracellular matrix
(4) activated LFA-1 binds to ICAM-1
(5) diapedesis

* dendritic cells' history
(1) exit marrow in immature state + enter circulation
(2) leave circulation + take station in peripheral tissues
(3) infection leads to intro + proliferation of pathogens
(4) contact antigen + receive activation signal via PAMPs
(5) transitions to activation + antigen processing
(6) matures + migrates via basement membrane to draining lymphatics + afferent lymph vessels
(7) antigen presentation to T cells

- vessels empty excess fluids into left subclavian vein via the thoracic duct

- Three T-Cell Activation Signals
  1) MHC/peptide, TCR
     → purpose is to transcriptionally activate the IL-2 gene
     → delivered by the peptide: MHC complex sensed by TCR
  2) CD80/CD86, CD28
     → purpose is to amplify signal 1 via IL-2 mRNA stabilization
     → delivered through costimulatory molecules on APC via CD28 on the T cell
  3) soluble DC factors
     → purpose is to cause differentiation of proliferating + immature effector T cells into subtype
     → delivered by various interleukins + TGFs secreted from DCs + possibly other accessory cells
     i) IL-12 ⇒ T-bet ⇒ T_H1
     ii) IL-4 ⇒ GATA-3 ⇒ T_H2
     iii) IL-23, IL-6, IL-1β ⇒ ROR-γ ⇒ T_H17
     iv) TGF-β, IL-17 ⇒ FOXP3 ⇒ T_{REG}

- Signal 1 In-Depth
  1) MHC:peptide complex proximity leads to activation of src homology tyrosine kinases (Fyn + Lck) via phosphorylation of double-tyrosine motifs on ITAMs
  2) ITAM phosphorylation leads to ZAP-70 binding to zeta chain of CD3 complex, and in turn phosphorylates LAT + SLP-76, which form an active complex w/GADS
  3) GADS:SLP-76:LAT complex recruits phospholipase c (PLC-γ), which is phosphorylated/activated by Itk
  4) activated PLC catalyzes breakdown of membrane component PIP2 to DAG + inositol triphosphate (IP_3)
  5) IP_3 binds to IP_3 receptors on ER surface, triggering calcium release-activated calcium channels (CRAC), which increases intracellular [Ca^{2+}]
  6) calmodulin binding to Ca^{2+} exposes hydrophobic patches, allowing assoc. w/other proteins for regulatory purposes (binds 4 ions)
  7) binds to calcineurin phosphatase, dephosphorylating nuclear signal NFAT

- Signal 2 In-Depth
  1) diacyl glycerol component of *phosphatidyl inositol bisphosphate* initiates additional signaling paths via *PKC-B* + *RasGRP* binding
  2) costimulation between CD80/CD86 + CD28 on cell surface increases IL-2 mRNA stability + production
     → α chain for receptor is high-affinity for IL-2
- Signal 3 + T_H Subtypes
  1) T_H1
     → major driver is IL-12 signal
     → activates T-BET to produce IL-2 + IFN-γ
     → helps macrophages kill intracellular pathogens
       - done via CD40/CD40L signaling in combination w/ IFN-γ
       - activated T_H1 can produce factors aiding <u>intravesicular/cell-mediated immunity</u>
         - <u>granuloma</u>: entity forming upon incomplete elimination of intravesicular pathogen
     → "two-lock box"
       - DC require dual TLR ligands or IFN-γ + single TLR ligand to stimulate IL-12 induction
  2) T_H2
     → major driver is IL-4 signal
     → activates GATA-3 to produce IL-4 + IL-5
     → helps support antibody-mediated immunity
       - promotes class-switching in B cells
     → adapted for eliminating multicellular *helminths*
       - promotes IgE production via IL-4 + IL-5; IgE binding to antigen allows *histamine* release by *mast cells* at the infection site
       - activates *eosinophils* to disgorge contents onto helminths
  3) T_H17
     → major driver is IL-23 + IL-6 + IL-1β
     → activates ROR-γ to produce IL-17 + IL-22
     → strong responses
       - assoc. w/ clearance of bacterial infections established on mucosal surfaces
       - possible autoimmune contributor
  4) T_{REG}
     → major drivers are TGF-β + IL-17
     → activates FOXP3 to produce TGF-β + IL-10
     → thought to limit immune responses via *peripheral tolerance*
       - growing tumors may exploit this to inhibit anti-cancer immune responses

- CTL Activation + Principles
  - three pathways
    1) DCs express high B7 levels + activate naïve CD8 T cells; IL-2 secretion by cell drives own proliferation/differentiation
    2) APCs stimulate effector CD4 T cells which activate APC; activated APC expresses B7 to costimulate naïve CD8 T cells
    3) APC activates CD4 T cell to make IL-2 + express IL-2 receptors; secreted IL-2 bound by CD8 T cells
  - re-activation in periphery does not require B7 costimulation
  - see antigen in context of class I MHC
    → can interact w/any nucleated cell + therefore kill infected self cells
    → produce cytotoxins after secondary lymphoid activation
      - initially contact targets via ICAMs
      - granule contents disgorged upon peptide/ligand complexing w/class I MHC
- Mucosal Surfaces
  - <u>mucous</u>: thick fluid underlying glycoproteins, proteoglycans, peptides, + enzymes
    → protects underlying epithelial cells + control infection
      - immune system must respond differently due to vulnerability of tissue + <u>normal flora</u> presence
  - often assoc. w/secondary lymphoid tissues
    → <u>Waldeyer's ring</u>: tonsils + adenoids forming ring of secondary tissues around gut + airway entrance
    → <u>GALT</u>: gut-associated lymphoid tissues
      - includes the mesenteric lymph nodes + Pyer's patches in the lamina propria
        - patches often assoc. w/villi
      - <u>M cells</u>: "microfold" cells serving an antigen acquisition function among GALT
        - release antigens to DC at basal surface via <u>transcytosis</u>
  - populated w/intraepithelial lymphocytes
    → $CD8^{pos}$ T cells
    → immersion process
      i) naïve T + B enter Peyer's patches via HEV; attracted by CCL19 + CCL21 and sensed through CCR7
      ii) leave patches upon activation + cease CCR7 + L-selectin production
      iii) express α4:β7 integrin in blood, which binds to MadCAM-1 + guides back cells to mucosa along w/CCL25, which is sensed by CCR9 receptor
      iv) CCR9 + αE:β7 integrin interact w/E-selectin on enterocytes, allowing entrance into gut epithelium
    → initial mucosal-homing signal is retinoic acid
      - is a derivative of Vitamin A
  - served by two antibody classes
    1) IgM
    2) IgA
      i) IgA1 found in more sterile sites
      ii) IgA2 found in less sterile sites

- Mucosal Surfaces (cont.)
  - enterocytes are equipped w/cytoplasmic PAMPs (NOD proteins)
    - → bind muramyl tripeptide (Gram-) or muramyl dipeptide (Gram+)
      - oligomerize + activate RICK
      - RICK activates NF-κB, leading to cytokine, chemokine, + defensin production
- Secondary Immune Response
  - faster + stronger
    - → more antigen-specific memory cells around after infection than naïve cells beforehand
      - can become effector cells like Th subtypes, CTL, + antibody-secreting plasma cells
  - no IgM due to prevention of naïve B cells from participating in subsequent pathogen exposure
    - → FcγRIIB1 receptor + surface Ig bind pathogen
      - sends a negative signal to naïve B cells but not to memory
        - ◊ property exploited in treating Rh incompatability to prevent fetal hemolytic anemia
- Distinguishing T cells
  - protein profile chart:

	naïve T cells	effector T cells	memory T cells
CD45RO	+	+++	+++
CD45RA	+++	+	−
CD62L (L-selectin)	+++	−	(some) +++
CCR7	+++	+/−	(some) +++
IFN-γ	−	+++	+++
Granzyme B	−	+++	+/−
FasL	−	+++	+

  - central memory cells
    - → express CCR7 + CD62
    - → reside in lymph nodes + await DCs
  - effector memory cells
    - → express CCR3 + CCR5
    - → patrol peripheral tissues to be first on site in case of infection
- NK Cells
  - lack TCR + CD3 but have identifying markers CD56 + NKG2D
  - sense abnormally low class I levels (common intracellular pathogen evasion mechanism)
    - → activated by low levels + stress proteins MIC-A/B
    - → non-polymorphic HLA-E binds to signal sequence on surface (HLA-A, B, C)
      - CD94: NKG2A detects HLA-E + inhibits NK cell (or KIR)
    - → suppression of HLA-A,B,C production by pathogen leads to HLA-E absence + NK activation

- NKT Cells
  - express both NK receptors + αβ TCR
    - → TCR repertoire allows for lipid/glycolipid infections presented by CD1d
      - DCs express CD1a + can present to NKT cells
  - produce IFN-γ that can activate DC for IL-12 production
    - → activates macrophages
    - → done by CD1d binding to self-glycolipids
- Pathogenic Terminology
  - agent: general term for a microbe causing disease
  - host: organism infected by a pathogen
    - → primary: parasite attains maturity + reproduces sexually
    - → secondary: harbors parasite for a short transition period
    - → dead-end: can become infected + develop disease but usually not transmit infection
    - → reservoir: long-term, natural host that may/may not display disease when infected but serve as a source of organisms to infect other species
  - vector: living organism that transmits an agent from one host to another
  - fomite: inanimate object that can become contaminated w/ an agent + facilitate transmission
  - transmission
    - → horizontal: passed from one individual to another w/in same generation/peer group
    - → vertical: passed from mother to fetus during pregnancy, development, or childbirth
    - → zoonosis: transmissible from animals to humans under natural conditions
    - → epizootic: affects an atypically high proportion of animals of the same species
- Immune Evasion Strategies
  1) breaching anatomical barriers
     - → ex. American hookworm
     - → ex. schistosomiasis
     - → ex. vector-borne diseases
       - kissing bug ⇒ chagas disease
       - mosquito ⇒ malaria ⇒ yellow fever ⇒ dengue ⇒ encephalitis
       - flea ⇒ plague
       - louse ⇒ typhus ⇒ Ricketts
       - sandfly ⇒ Leishmaniasis
  2) speed (replicate + transmit before adaptive immune system kicks in)
     - → ex. norovirus
     - → ex. cholera
  3) evading consequences of phagocytosis
     - → ex. listeriosis (utilizes listeriolysin O to lyse phagosome membrane)
     - → ex. tuberculosis (survives in endocytic vesicles five ways)
       - i) prevent phagosome maturation
       - ii) block phagosome/lysosome acidification
       - iii) resist reactive oxygen intermediates
       - iv) recruitment of tryptophan-aspartate containing coat protein
       - v) virulence proteins

- Immune Evasion Strategies (cont.)

4) stable strain variability
   → ex. streptococcus pneumoniae
      - serotypes: variations in sugar combinations on capsules

5) quick antigenic changes
   → ex. Influenza A
      - antigenic drift: lack of fidelity copying genome leading to primary structure changes that can neutralize antibodies from a previous infection
      - antigenic shift: superinfection of two different strands leads to shuffling of gene segments in progeny viruses
   → ex: Trypanosoma brucei ("parasite w/1000 faces")

6) stealth
   → ex. malaria (RBC cloak)
   → ex. adenovirus/dsDNA viruses (interfere w/MHC transport + production)

7) subversion (passing false signals)
   → ex. EBV (infects B cells + produces IL-10-like protein)

- Immunodeficiencies
  - primary: caused by genetic defects
    1) recessive
    2) dominant
    3) X-linked

    } can be through receptor deficiencies, defects in antibody production (ex. XLA + Hyper IgM), defects in phagocytes (ex. CGD), defects in T cells (ex. class II Bare Lymphocyte Syndrome, ADA deficiency)
  - acquired: caused by environmental factors or other disease
    → ex. HIV (four ways it defeats the immune system)
      i) attacks helper T cells
      ii) Nef downregulates MHC (stealth)
      iii) Rev gene lacks proofreading, leading to mutations that the immune system cannot immediately see
      iv) does not need to enter serum to infect new cells

- Hypersensitivity Response Types

1) Type I: caused by generation of IgE that binds to F_c receptors on mast cells
   → mast cells release products from pre-formed granules (including histamine) and synthesize other products for later release
      - histamine increases vascular permeability + smooth muscle contraction
   → basophils + eosinophils also express $F_c\varepsilon RI$
      - both release granular products
      - eosinophils secrete IL-5 + basophils IL-4/IL-13

2) Type II: caused by small molecules attaching to + altering self-protein
   → mediated by IgM + IgG
   → tends to be to RBCs or platelets
      - altered self-protein processed + presented to T cells, driving Ab response
      - Ab produced destroys cells via complement-mediated lysis +/or F_c-mediated phagocytosis

3) Type III: immune complex disease caused by Ab generation against small, soluble proteins
   → medium-sized complexes deposit in vessels + fix complement

4) Type IV: "delayed type" caused same way as II but mediated by T_H + CTL

α4:β7 integrin	binds to *MadCAM-1* and guides B/T cells back to mucosa w/*CCL25* and sensed by *CCR9 receptor*
αE:β7 integrin	interacts w/*E-selectin* on enterocytes and allows entrance into gut epithelium
B7	molecules expressed by DC to activate naïve CD8 T cells
CCL19	factor attracting naïve T/B cells to Pyer's patches along w/*CCL21* and sensed by *CCR7*
CCL21	factor attracting naïve T/B cells to Pyer's patches along w/*CCL19* and sensed by *CCR7*
CCL25	guides T/B cells back to mucosa along w/ *a4:b7 integrin* bound to *MadCAM-1*
CCR3	distinguishing factor expressed by effector cells along w/*CCR5*
CCR5	distinguishing factor expressed by effector cells along w/*CCR3*
CCR7	distinguishing factor expressed by memory cells along w/*CD62*
CD1a	factor expressed by DC allowing presentation to NKT cells
CD34	molecule allowing rolling interactions along w/*GlyCAM-1*
CD40	helps activate macrophages along w/*IFN-Y;* produced by Th1
CD45RA	factor expressed heavily by naïve T cells but only slightly on effector and not on memory
CD45RO	factor expressed heavily by effector and memory T cells but only slightly on naïve
CD56	identifying marker for NK cells along w/*NKG2D*
CD62	distinguishing factor expressed by memory cells along w/*CCR7*
CD62L	cell adhesion molecule found on lymphocytes
CRAC	channels opening upon *IP3* binding and increasing intracellular [Ca2+]
DAG	breakdown product of *PIP2* initiating Signal 2 w/*PKC-B* and *RasGRP to stabilize IL-2* mRNA
E-selectin	cell adhesion molecule expressed only on endothelial cells
FasL	expressed heavily by effector cells and lightly by memory cells but not by naïve T cells
FceRI	expressed by basophils and eosinophils
FcyIIB1	prevents naïve B cell activation during secondary immune response along w/surface Ig
FOXP3	gene activated by *TGF-B* and *IL-17* to produce more *TGF-B* and *IL-10* and drive Treg production
Fyn	*SRC homology tyrosine kinase* phosphorylating *ITAM* motifs w/*Lck*
GATA-3	gene activated by *IL-4* to produce *IL-4* and *IL-5* to drive Th2 formation
GlyCAM-1	molecule allowing rolling interactions along w/*CD34*
HLA-E	binds to signal sequences and keeps NK cells inactive; absence leads to NKT activation
ICAM	intercellular adhesion molecule
IFN-Y	macrophage activator; found heavily on effector and memory cells but not naïve T cells
IgA1	more flexible IgA found in sterile sites of mucosal surfaces
IgA2	less flexible IgA found in less sterile sites of mucosal surfaces
IL-10	similar molecule secreted by EBV in order to provide false signals to immune system

IL-12	interleukin activating *Th1* through *T-bet*
IL-13	interleukin secreted by basophils along w/*IL-4*
IL-17	interleukin activating *Treg* through *FOXP3* w/*TGF-B*
IL-1B	interleukin activating *Th17* through *ROR-Y* w/*IL-6* and *IL-23*
IL-2	interleukin driving Th1 formation after *T-bet* activation; also helps activate CTLs
IL-23	interleukin activating *Th17* through *ROR-Y* w/*IL-6* and *IL-1B*
IL-4	interleukin activating *Th2* through *GATA-3*
IL-4	interleukin secreted by basophils along w/*IL-13*
IL-5	interleukin secreted by eosinophils
IL-6	interleukin activating *Th17* through *ROR-Y* w/*IL-23* and *IL-1B*
IP3	*PIP2* breakdown product triggering *CRAC* channels and increasing [Ca2+]
ITAM	double-tyrosine motifs on *SRC homology tyrosine kinases* that are phosphorylated by *Fyn* and *Lck*
KIR	can take the place of *CD94:NKG2A* complex and keep NK cells inactive
LAT	forms an active complex during Signal 1 transduction w/*GADS* and *SLP-76*
Lck	*SRC homology tyrosine kinase* phosphorylating *ITAM* motifs w/*Fyn*
LFA-1	activated form binds *ICAM-1* and stops rolling interaction
MIC-A/B	stress proteins found alongside low MHC I levels triggering NK activation
NFAT	nuclear transcription signal in Signal 1 activated by dephosphorylation by calcineurin phosphatase
NKG2D	identifier for NK cells along w/*CD56*
PIP2	membrane component broken down into *DAG* and *IP3* by activated *PLC-Y*
PKC-B	helps initiate Signal 2 along w/*RasGRP* via *DAG* activation
PLC-γ	activated by *GADS:SLP-76:LAT complex* and catalyzes *PIP2* breakdown to *DAG* and *IP3* in Signal 1 transduction
RasGRP	helps initiate Signal 2 along w/*PKC-B* via *DAG* activation
RICK	activates *NF-kB* through *NOD proteins* to produce cytokines, chemokines, and defensins
ROR-Y	gene activated by *Il-23*, *IL-6*, and *IL-1B* to produce *IL-17* and *IL-23* to drive Th17 production
SLP-76	forms an active complex during Signal 1 transduction w/*GADS* and *LAT*
T-bet	gene activated to produce *IL-2* and *IFN-Y* and drive Th1 production
TGF-B	transforming growth factor activating *Treg* through *FOXP3* w/*IL-17*
ZAP-70	factor binding upon *ITAM* phosphorylation in Signal 1 and phosphorylating *LAT* and *SLP-76*