

Transplant Immunology

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Disclosures

- The author of this presentation has no actual or potential conflicts of interest



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Learning Objectives

1. Differentiate between components of innate and adaptive immunity.
2. Review lymphocyte activation, differentiation and effect mechanisms.
3. Discuss pathways of allorecognition, transplant rejection and injury.
4. Assess immunologic risk of rejection.
5. Describe pathways to immunologic tolerance of allograft.

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Immunity

Innate

- Nonspecific, rapid
 - Physical barriers: skin, tissue
 - Chemical barriers: complement, pathogen associated molecular patterns, damage associated molecular patterns (DAMPs)
- Mediated by various cells including mast cell, macrophages, neutrophils, dendritic cells, natural killer cells, etc.
- No immunological memory

Adaptive

- Antigen (Ag) specific, slower
 - Ag is any molecule that can activate adaptive immunity
- Mediated by lymphocytes and antigen presenting cells
- Exhibits immunological memory

Abbas AK, Lichtman AH and Pillai S, ed. Cellular and Molecular Immunology, Philadelphia, PA: Elsevier, 9th Ed, 2018.
 Coico R and Sunshine G, eds. Immunology A Short Course, Hoboken, NJ: Wiley-Blackwell, 7th Ed, 2015.

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Immunity

- Innate immune system (IMS) causes:
 - Allograft injury during reperfusion
 - Inflammation, scarring
 - Primes/enhances activity of the adaptive IMS
- Adaptive IMS is responsible for
 - Allograft recognition
 - Rejection
 - Tolerance

Although distinct, closely interconnected and depend on each other for activation and attenuation.

Also, not mutually exclusive.

Abbas AK, Lichtman AHH and Pillai S, ed. Cellular and Molecular Immunology, Philadelphia, PA: Elsevier, 9th Ed, 2018.
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Ubiquitous Role of Cytokines

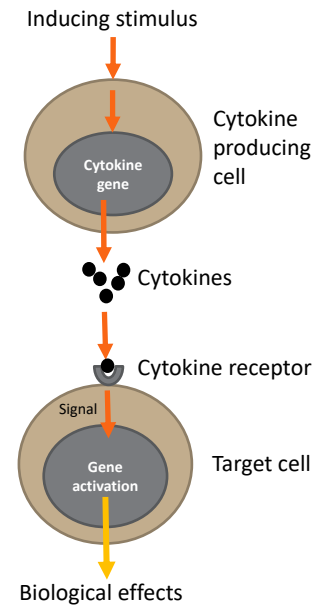
- Chemical messengers
 - Mediate signaling and communication between cells
 - Guide the behavior of cell; its response to environmental changes
- Produced on demand by various cells
 - “Inter-leukins” (IL) misnomer
 - Secretion is brief, self-limited event in response to a stimulus
- Major function
 - Inflammatory responses (TNF-alpha, IL-1, IL-6, IL-17, chemochines)
 - Proliferation, differentiation of cells (IL-2, IL-4, IL-5, IL-10, IL-12, INF-gamma, TGF-beta)
 - Neuronal, hematopoietic and embryonic development (erythropoietin, thrombopoietin, GCSF, etc.)

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Cytokines

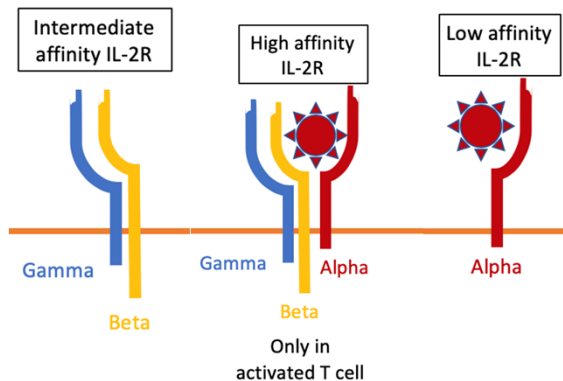
- Act only on cells bearing specific membrane receptors
 - Cytokine binding unit
 - Signal transduction unit (often shared by various cytokines resulting in redundancy and pleiotropy)
- Regulation of cytokine function occurs by...
 - controlling quantity of cytokine produced/available
 - controlling expression of cytokine receptor components



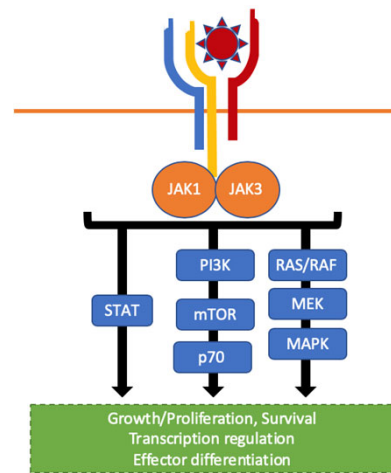
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IL2 Receptor



- Beta/Gamma signal transduction unit exists on resting cells
- Alpha subunit, a.k.a CD25 is the cytokine binding site
- It is produced by active lymphocytes only



Cellular responses consist of changes in gene expression and cell function

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Case Question 1

Cytokine release syndrome is a complication often associated with the use of lymphocyte depleting agents such as alemtuzumab (MS, leukemia/lymphoma, transplant) or anti-thymocyte globulin (transplant). This complication is considered a “first dose effect” resulting from a massive release of cytokines from lysing cells. Patients will often present with fevers, sweating, malaise and hypotension. Which cytokine is most likely responsible for these symptoms?

- A. IL-2
- B. IL-12
- C. TGF-beta
- D. TNF-alpha

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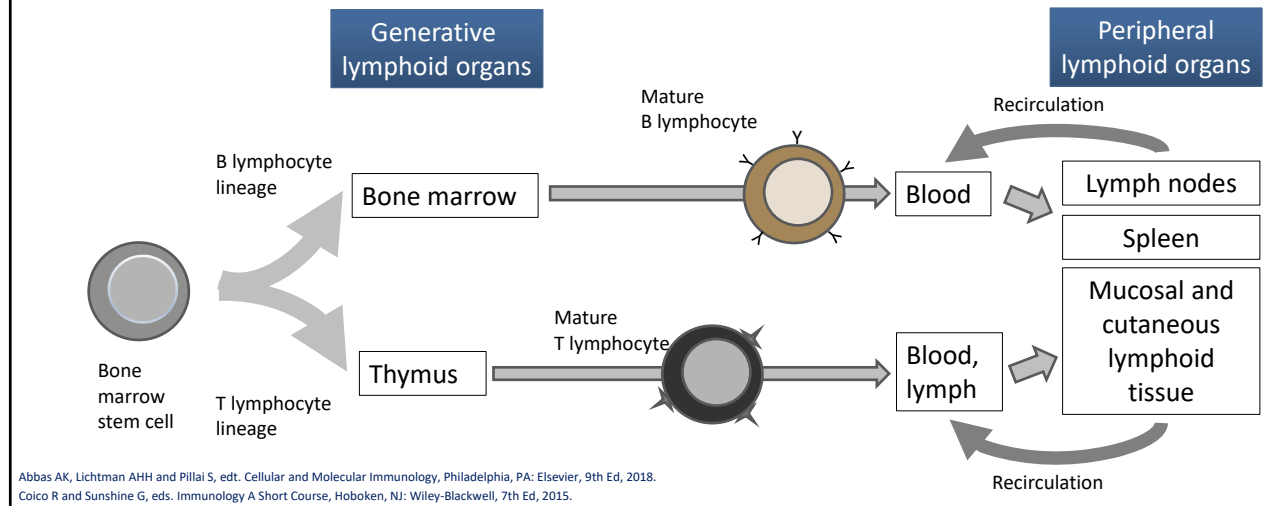
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- C. TGF-beta
- D. TNF-alpha

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Lymphocytes



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Lymphocytes

- Naïve lymphocytes
 - Mature lymphocytes that predominantly reside in lymph nodes
 - Function → Ag recognition
 - T Cells cannot recognize Ag on their own
 - B cells can recognize Ag on their own
- Effector lymphocytes
 - Ag activated lymphocytes that eliminate pathogen (humoral vs. cellular responses)
 - Undergo rapid clonal expansion of Ag specific cells (~7-14 days)
- Memory lymphocytes
 - Long-lived, functionally silent cells that survive clonal decline
 - Mount rapid and robust response to antigen (secondary responses; 3-5 days)
 - Phenotypically different (express high level of integrins and chemokine receptors)

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Lymphocytes

Cell	Effector Function	Phenotypic Markers
Helper T cells (Th)	<ul style="list-style-type: none"> Cytokine production → instruct cells to do their job (T-cell differentiation, B-cell activation, macrophage activation, etc.) Cellular or humoral response MHC class II restricted activation 	CD3 a.k.a. TCR CD4
Cytotoxic T cells (Tc)	<ul style="list-style-type: none"> Lysis of virus and tumor infected cells; allograft cells Cellular response MHC class I restricted activation 	CD3 a.k.a. TCR CD8
B cells	<ul style="list-style-type: none"> Antibody production (humoral response) 	CD19 + CD21 a.k.a. BCR MHC I and II

CD – cluster of differentiation; TCR – T cell receptor
 BCR – B cell receptor;
 MHC – major histocompatibility complex

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Major Histocompatibility Complex (MHC)

- Region on chromosome VI that gives rise to Human Leukocyte Antigens (HLA)
 - Proteins that are essential for function of our adaptive IMS
- Function → antigen presentation to T cells
- Two major HLA classes:
 - Class I expressed on nearly all nucleated cells → interact with Tc cells
 - Class II exist on specialized antigen presenting cells → interact with Th cells
- Allograft rejection responses are due to differences in HLA between donor and recipient
 - Mismatched, non-self-Ag serve as targets for adaptive immune system

Womer, K., & Rabb, H. (2010). Immunologic Principles in Kidney Transplantation. In Comprehensive Clinical Nephrology (pp. 1119-1133).

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HLA Matching

- Every human has 6 distinct sets of HLA protein
 - MHC Class I: HLA-A, HLA-B, HLA-C
 - MHC Class II: HLA-DP, HLA-DQ, HLA-DR
- We possess two alleles for every HLA protein ($\frac{1}{2}$ inherited from each parent)
 - Identical twins match all 12 HLA proteins
 - Non-identical siblings carry 25% chance for identical HLA match
- In organ transplantation, 6/6 HLA antigen match or 0/6 mismatch is ideal
 - For organ transplantation, only HLA A, B and DR <52 are matched
 - HLA C, DP and DQ → considered less immunogenic and ubiquitous

Womer, K., & Rabb, H. (2010). Immunologic Principles in Kidney Transplantation. In Comprehensive Clinical Nephrology (pp. 1119-1133).

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Case Question 2

What is the degree of HLA mismatch considered relevant in solid organ transplantation between these two patients?

Patient 1

A 1, 12
 B 13, 14
 C 4, 27
 DP 15, 22
 DQ 14, 18
 DR 11, 21

Patient2

A 1, 26
 B 14, 18
 C 4, 36
 DP 15, 22
 DQ 2, 14
 DR 14, 19

- A. 1/6
 B. 2/6
 C. 4/6
 D. 5/6

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Case Question 2

What is the degree of HLA mismatch considered relevant in solid organ transplantation between these two patients?

Patient 1

A 1, 12
B 13, 14
C 4, 27
DP 15, 22
DQ 14, 18
DR 11, 21

Patient2

A 1, 26
B 14, 18
C 4, 36
DP 15, 22
DQ 2, 14
DR 14, 19

- A. 1/6
- B. 2/6
- C. 4/6**
- D. 5/6

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Antigen Presenting Cell (APC)

- Cells that besides MHC-I, can also display MHC-II associated Ag
 - Dendritic cells, macrophages and B cells
 - FYI: during extreme duress, vascular endothelial cells can also display MHC-II but are not mobile and not considered APCs
- Function
 - Process and present peptide antigen to Th cells
 - Provide secondary stimuli to Th cell required for full Th cell response

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Antigen Presenting Cells

- Most efficient at initiating response in lymph node
- Broadest range of antigen presentation
- High level of MHC, costimulatory and adhesion molecules

- Primary action in periphery
- Provide continuous stimuli to maintain cellular response
- Low [MHC II] at baseline/high [MHC II] after cytokine stimulus

- Have high affinity for Ag and rapidly processes Ag onto MHC (30 min)
- Important in immunologic memory or later stages of primary infection

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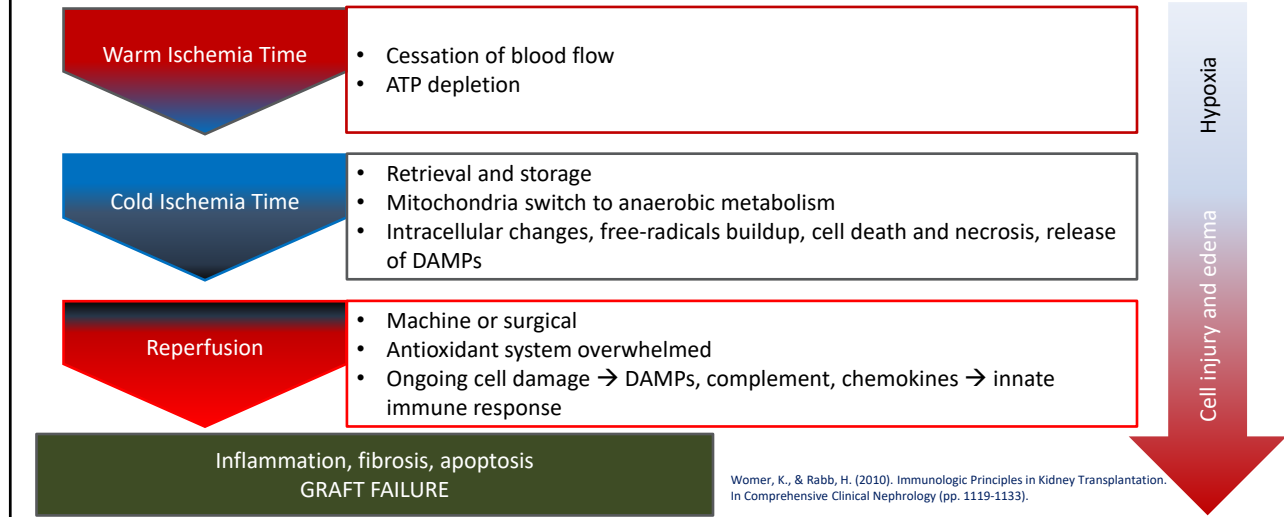
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INNATE IMS MECHANISMS OF INJURY TO THE ALLOGRAFT

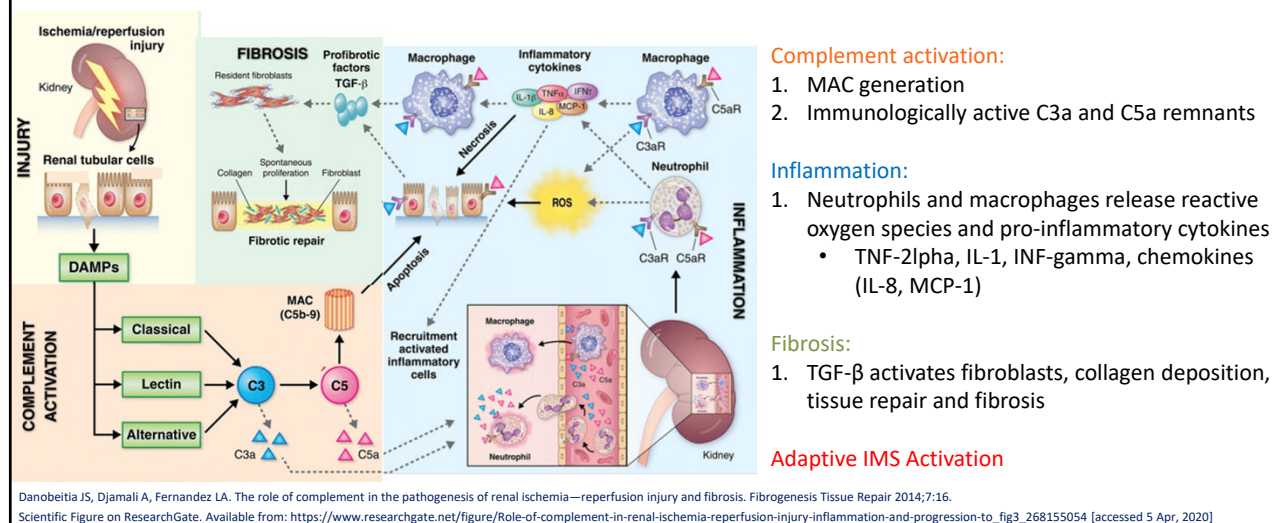
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Innate IMS: Ischemia Reperfusion Injury




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Innate IMS: Ischemia Reperfusion Injury

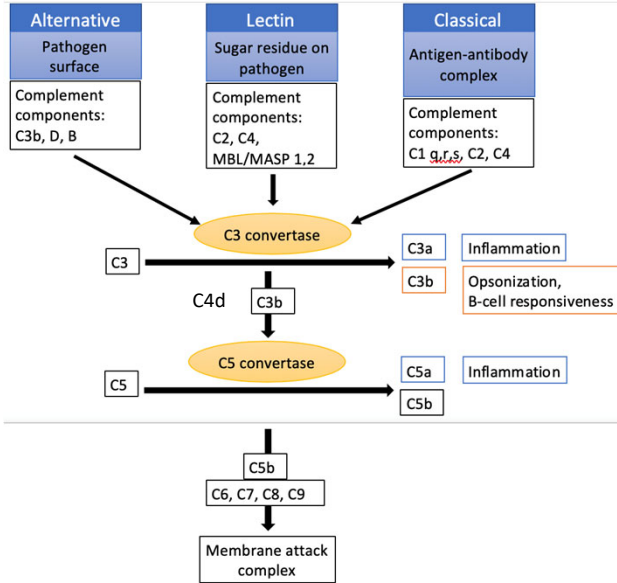


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Complement

- Soluble and membrane bound proteins
- Major function:
 - Lyses cells by membrane attack complex
 - Forms anaphylatoxin → inflammation
 - Opsonizes toxins, pathogens, antigen source
 - Enhances B-cell response
- Immunologically inert metabolite C4d may be used for rejection monitoring in tissue biopsies




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graph TD
    subgraph Pathways
        A[Alternative Pathogen surface  
C3b, D, B] --> C3C
        L[Lectin Sugar residue on pathogen  
C2, C4, MBL/MASP 1,2] --> C3C
        C[Classical Antigen-antibody complex  
C1, C2, C3, C4] --> C3C
    end
    C3C((C3 convertase)) --> C3[C3]
    C3C --> C3a[C3a]
    C3C --> C3b[C3b]
    C3b --> C4d[C4d]
    C3b --> C5C((C5 convertase))
    C5C --> C5[C5]
    C5C --> C5a[C5a]
    C5C --> C5b[C5b]
    C5b --> C6C7C8C9[C6, C7, C8, C9]
    C6C7C8C9 --> MAC[Membrane attack complex]
  
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Complement Blockade Implications

- Complement inhibitors downstream of C5 will prevent MAC formation
 - Does not prevent generation of immunologically active C3 byproducts that contribute to inflammation, and tissue injury
 - Also, since it blocks the terminal step, it will affect all three activation pathways creating high potential for infectious complications
- Inhibition of C1 only, will spare the lectin and alternative pathways
 - Also blocks generation of C3 and C5 byproducts
 - Potentially avert infectious complications
- Inhibition of C3 convertase

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Adaptive IMS: Ischemia Reperfusion Injury

- Exposure to allo-Ag:
 - Lysing allograft cells; both MHCI and MHCII Ag expressed on endothelial cells
- T cell activation in lymph nodes
 - Activated T cells infiltrate allograft
- Combined actions of lymphocytes, neutrophils and macrophages create microvascular sludging and 'no-flow' characteristic of IRI

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T CELL ACTIVATION

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T Cell Recognition Phase

- After APC processes Ag, it migrates to nearest draining lymph node
 - Dendritic cell can process 500 T cells/hr
 - Matching continues for 8-10 hours after exposure
- In the lymph node, APC-MHC-Ag interacts with the specific T Cell Receptor (TCR) complex on the surface of the T cell
 - TCR (a.k.a. CD3) interacts with Ag peptide bound to MHC molecule
- Co-receptor CD4 moves closer to TCR to stabilize interaction

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Is That All It Takes?

- Contact between TCR and APC is necessary but not enough
 - Low affinity interaction, cells are constantly moving, interacting components dissociate rapidly
 - TCR surface molecules have short cytoplasmic tails → cannot propagate Ag signal
- Sets of paired interactions between APC and T cell surface are required for full T cell activation
 - Costimulatory pairs
 - Adhesion molecules
- Interactions form a stable immunological synapse or a communication junction

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Paired Surface Interactions – Costimulatory Pairs

- Essential for activation of naïve T cells
 - Cluster intracellular signaling molecules around the TCR allowing activation signal to be dispatched to the nucleus
- Many pairs identified (APC/T cell):

– B7/CD28	CD137L/CD137 aka OX40/4-1 BB
– CD40/CD40L (CD154)	CD134L/CD134
– CD70/CD27	TIM-4/TIM-1
- Inhibition of pair binding impairs T cell response

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Paired Surface Interactions – Costimulatory Pairs

- | | |
|--|---|
| <ul style="list-style-type: none"> • B7/CD28 binding <ul style="list-style-type: none"> – Mobilizes kinases and other signaling proteins around TCR – Increasing half-life of cytokine mRNA molecules → 100 times more cytokines produced – Increases T-Cell proliferation – Prevents the induction of anergy and cell death <ul style="list-style-type: none"> – Induces expression of antiapoptotic proteins | <ul style="list-style-type: none"> • CD40/CD40L - Important for B cell activation <ul style="list-style-type: none"> – Promotes B cell proliferation – Promotes somatic hypermutation/affinity maturation – Required for Ab “class switch” – Promotes memory B cell formation – Promotes up-regulation of MHC and B7 protein |
|--|---|

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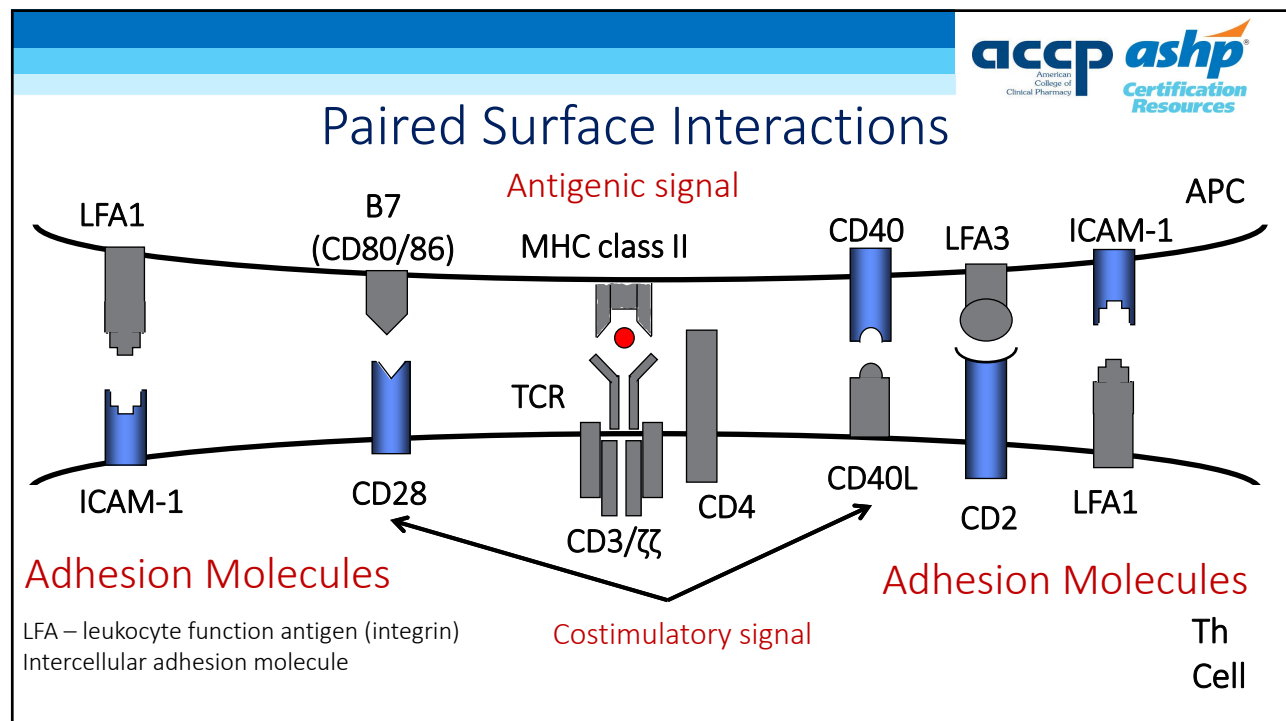
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Paired Surface Interactions – Adhesion Molecules

- Not specific to T cells or APC
- Adhesion molecules form an outer ring, stabilizing the T-cell–APC contact and assist in migration of T cell to sites of inflammation and infection
- Major adhesion molecules:
 - LFA-1(integrin)/CD54 (ICAM-1) or CD50 (ICAM-3)
 - LFA-3 (integrin)/CD2

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Case Question 3

A clinical trial investigating efficacy of a humanized anti-CD28 monoclonal antibody in prolonging kidney allograft survival shows that patients treated with this biologic agent have significantly fewer episodes of chronic rejection. Probable mechanism responsible for this effect involves:

- A. Blocking of CD28 binding to B7 protein interferes with the co-stimulation of T cell
- B. Blocking of CD28 interferes with the adhesion of T cell to antigen presenting cell
- C. Blocking of CD28 binding to CD3 interferes with the transmission of antigenic signal
- D. Blocking of CD28 binding to CD28L interferes with the co-stimulation of T cell

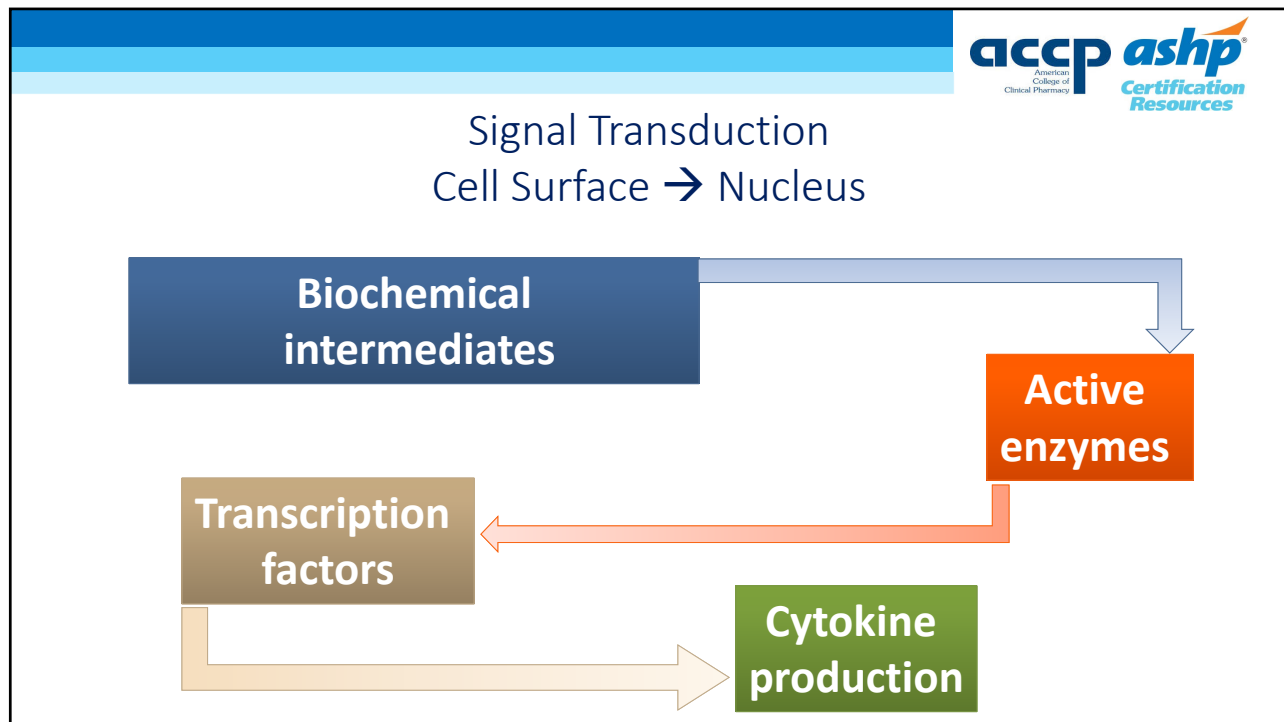
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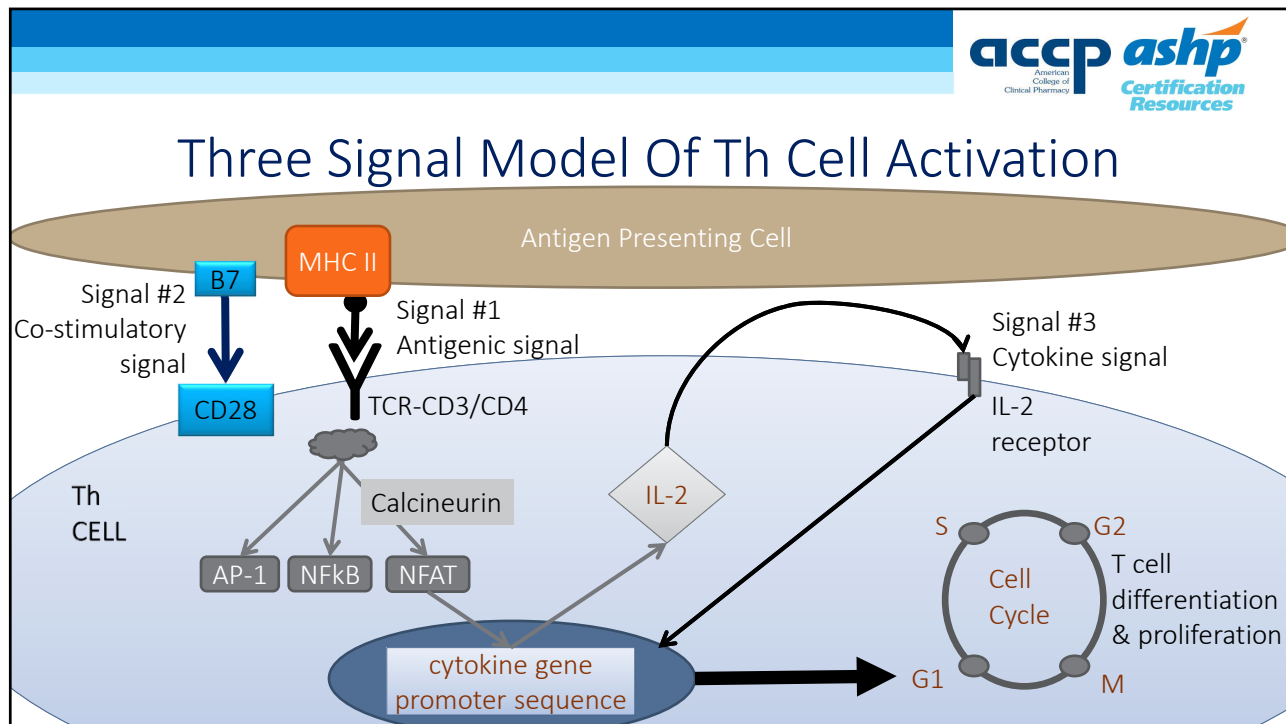
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Signal Transduction – Three Major Pathways

Pathway	Biochemical Intermediates	Enzyme	Transcription Factors
1	PLC-γ activation → PIP2 → IP3 → ↑Ca	Calcineurin	NFAT
2	PLC-γ activation → PIP2 → DAG	PKC	NF- κB
3	GDP/GTP exchange on Ras/Rac → Ras/Rac-GTP	ERK, JNK	AP-1

ZAP-70 – Zeta-chain-associated protein kinase 70;
 PLCγ – phospholipase C gamma; Ca – calcium; NFAT – nuclear factor of activated T cell;
 PIP2 – Phosphatidylinositol biphosphate; IP3 – inositol triphosphate; DAG – diacylglycerol;
 PKC – protein kinase C; NF-κB – nuclear factor κB; GTP – guanosine triphosphate;
 ERK – extracellular receptor-activated kinase; JNK – c-Jun N-terminal kinase; AP-1 – activation protein 1

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T Cell Activation Phase: Proliferation

- Activated Th cell secretes its own growth promoting cytokine (IL-2) and expresses cell surface receptor for that cytokine (IL-2 Receptor or IL-2R)
- During clonal expansion
 - Active cell proliferates to build up clones with same Ag specificity
 - T cell doubles its number every 6 hours for 3-4 days
- Activated T cell down regulates expression of homing molecules and upregulates expression of chemokine receptors and adhesion molecules that allow it to migrate out of lymph node and travel to affected tissue
- Following these events, differentiation occurs

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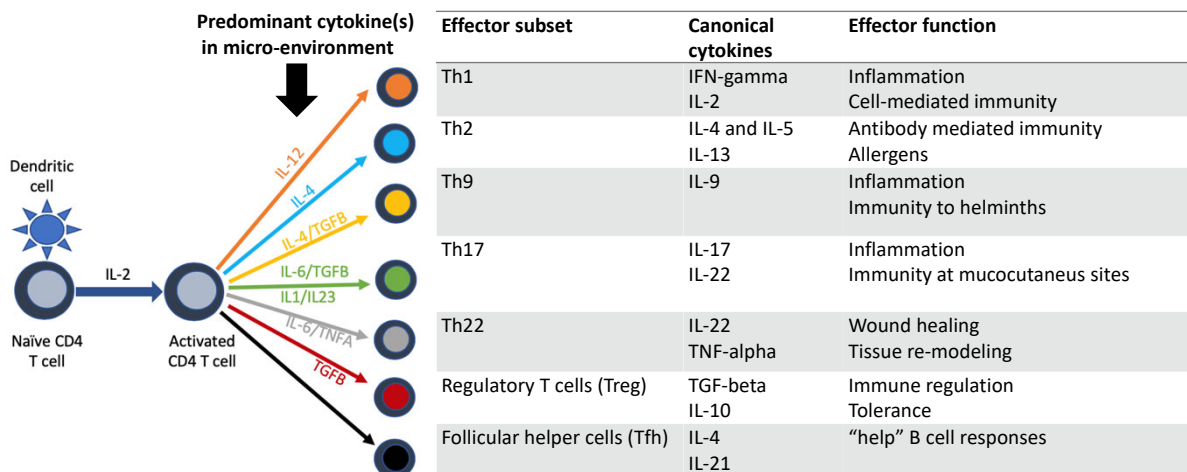
T Cell Activation Phase: Differentiation

- Conversion of naïve T cell to effector cell is called differentiation
 - How does Th cell know which cytokines to produce?
- Presence of cytokines in the microenvironment at the time of Ag recognition holds a key to Th cell differentiation
 - Activation of “lineage-determining” transcription factors

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Th Effector Phase: Cytokine Production



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Case Question 4

Which of the following statements about the activation of CD4+ T cell is INCORRECT?

- A. Activated cell synthesizes cytokines
- B. Interaction of B7 and CD28 is also known as the second signal
- C. Only peptide bound to the MHC class II activates the CD4+ T cell
- D. Activated calcineurin enters the nucleus, where it binds and activates IL-2 gene promoters

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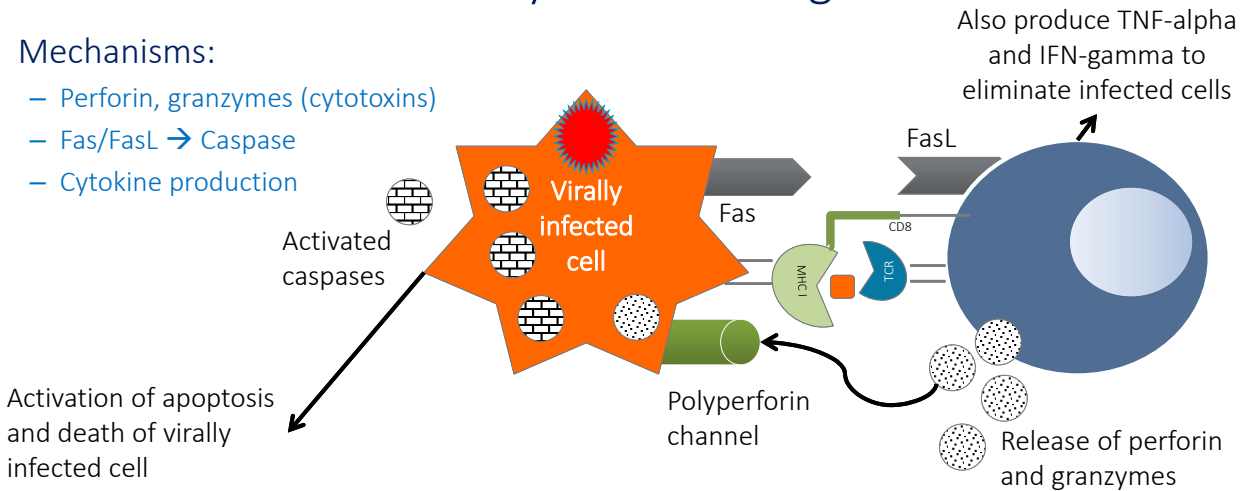
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Tc Effector Phase: Cytotoxic Killing Mechanisms

- Mechanisms:

- Perforin, granzymes (cytotoxins)
- Fas/FasL → Caspase
- Cytokine production



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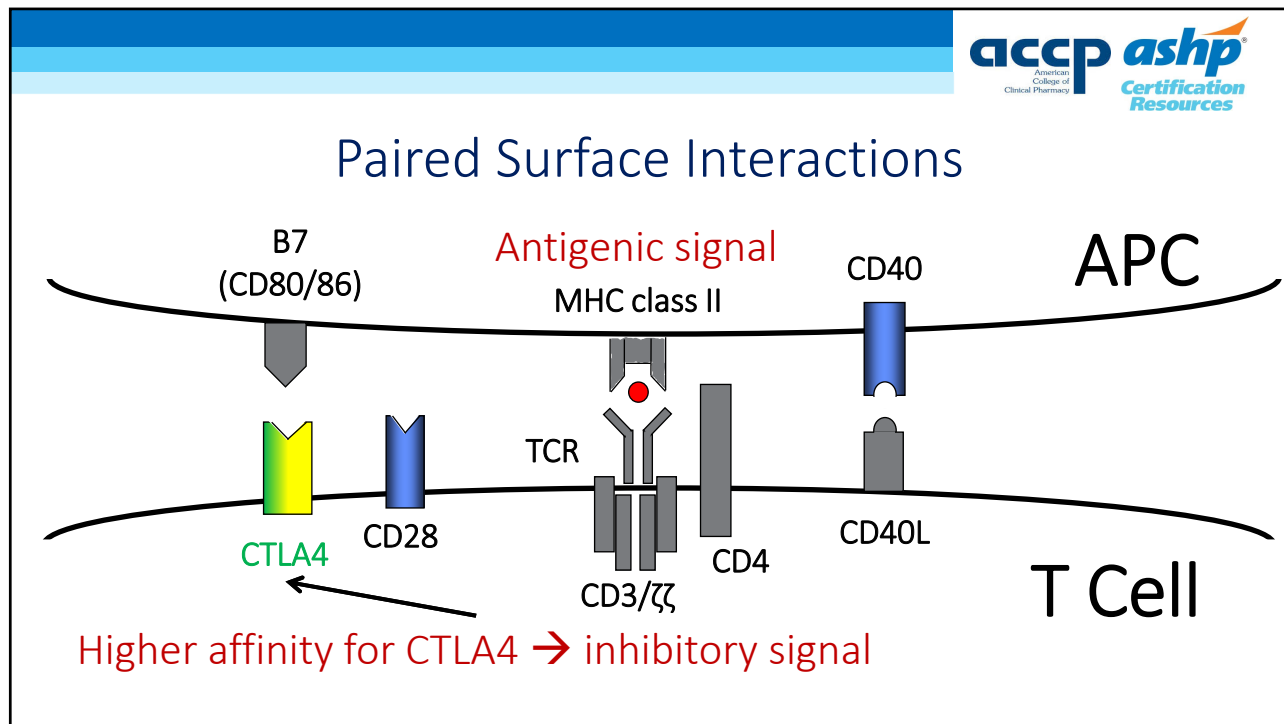
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T Cell Decline Phase: Negative Regulation

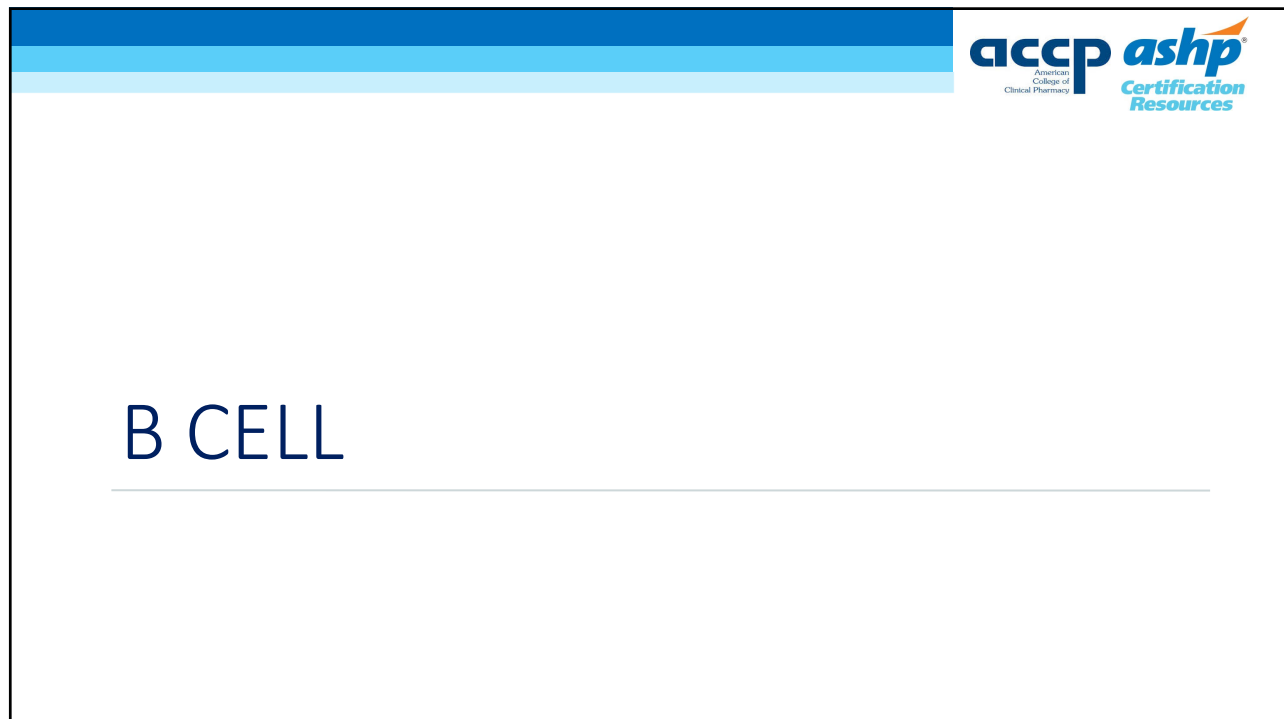
- 95% of effector T cells die via apoptosis
- Negative/inhibitory signals transmitted by several protein/receptor pairs
 - CTLA4 (Cytotoxic T-Lymphocyte Antigen-4) via B7 protein binding serves as a natural inhibitor and leads to memory cell generation w/in 24-48hours of activation
 - SHP2 (SH2-containing Protein tyrosine Phosphatase-2) via an ITIM (Immunoreceptor Tyrosine-based Inhibition Motif)
 - Programmed cell death protein (PD-1) is expressed on activated CD4+ and CD8+ and inhibits ZAP-70 function

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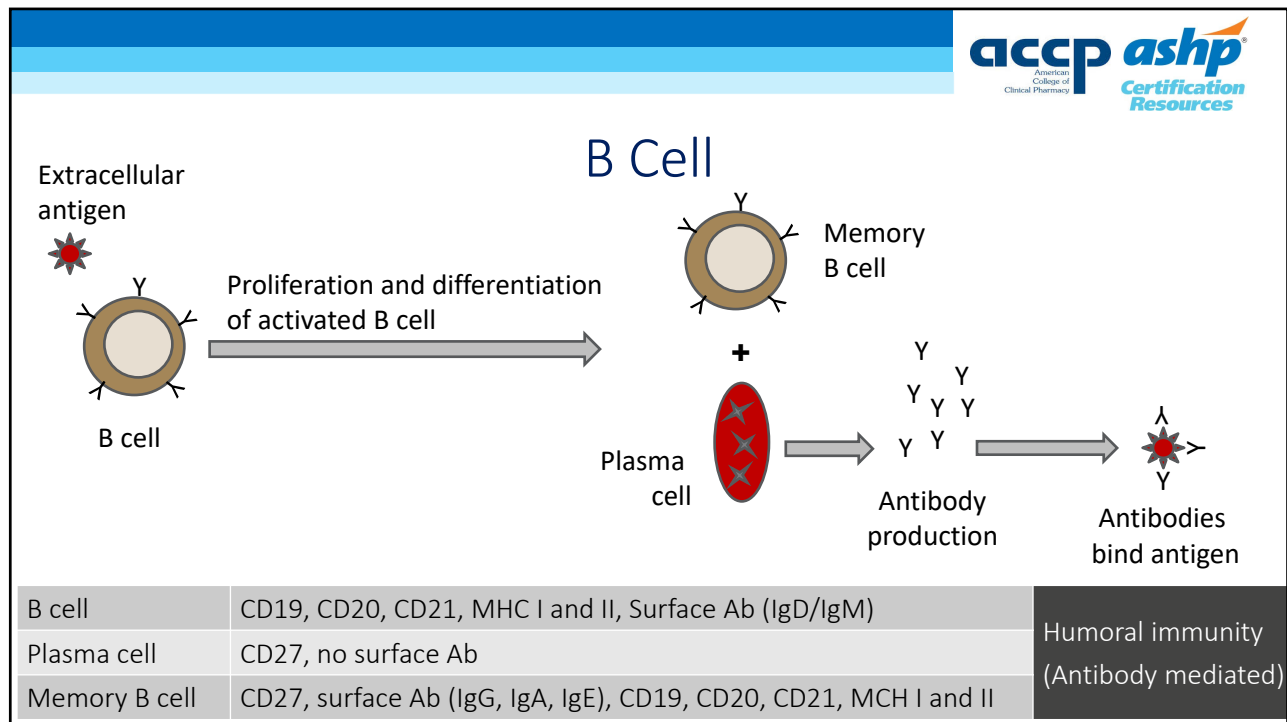
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Naïve B Cell Review

- In bone marrow, naïve B cell selects B-Cell Receptor (BCR) it will display on its surface
- BCR is the Ag recognition unit in the form of membrane bound antibody (Ig)
 - BCR of naïve B cell is either IgM or IgD
 - B cell has ~1,000 identical BCR on its surface → all recognize same Ag
- Following activation, through changes in variable and constant domains, Ab function and Ag specificity may be modified

Antigen binding site

Variable domains → antigen binding sites Fab

Constant domains → modulate immune activity Fc

Disulfide bridge

Light chain

Heavy chain

B cell plasma membrane

Signal transduction region

Ig β

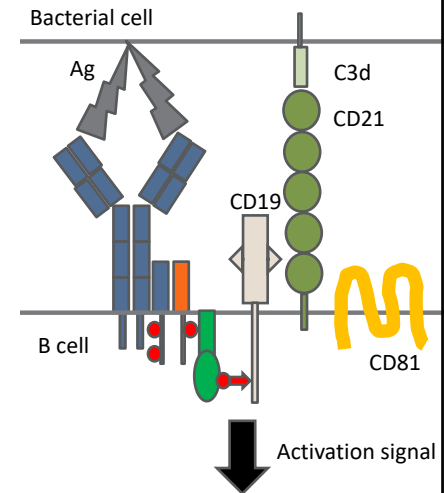
Ig α

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Rye, Connie, Robert Wise, Vladimir Jurukovski, Jean DeSaix, Jung Choi, and Yael Avissar. *Biology*. Houston, TX: OpenStax, 2016. <https://openstax.org/books/biology/pages/42-2-adaptive-immune-response>.

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BCR and Coreceptor Complex

- Coreceptor complex modulates BCR signal transduction
- Composed of CD81, CD21 and CD19
 - CD21 binds opsonized antigenic particles
 - CD19/CD81 are primarily responsible for signal transduction
- Complement C3d enhances B cell signaling and activation



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Recognition Phase – Naïve B Cell

- Naïve B cells reside in lymphoid organs where it may encounter an Ag specific for it's unique BCR
 - Ag is any organic molecule
 - B cell is a phagocyte
- More than 99% naïve B cells spend their entire life span without encountering Ag and die within few days
- What happens when B cell encounter Ag?

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Naïve B Cell Activation Phase

- Chemical structure of Ag critically influences the type of immune response that will be produced
 - Thymus independent (TI) Ag – polymeric Ag that crosslink the BCR and directly activate B cell
 - Only IgM produced
 - No memory
 - Thymus-dependent (TD) Ag – protein Ag that require activation guided by the Th cells
 - Produces IgM followed by IgG, IgE or IgA
 - Memory cells produced

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TD Ag B Cell Activation

- Following BCR ligation, decision between activation and remaining inert will depend on amount, avidity and timing of interaction and by the nature and amount of costimulation present
 - B7/CD28
 - CD40/CD40L (CD154)
- In the absence of properly timed interaction with a Th cell, the B cell remains inert or tolerant to Ag

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TD Ag B Cell Maturation Events

- Differentiation into Ab producing plasma cell or memory B cell
- Class switching
 - Changes the class (and function) of the antibody but not Ag specificity (Fab region unchanged, Fc region changes)
- Somatic hypermutation → affinity maturation
 - Changes the affinity of BCR to Ag (Fab region changes, Fc region unchanged)
 - Fine tuned BCR has higher affinity for cognate Ag

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TD Ag B Cell Secondary Response

- Primary response
 - APC such as Dendritic cell → Th cell → B cell → Antibodies
 - Occurs 5-15 days after exposure
- Secondary response (requires previous exposure to antigen)
 - Memory B cell (MHC II) → Th cell → Antibodies
 - Memory B cells have higher affinity for Ag due to somatic hypermutation → respond more rapidly → generate more progeny → produce higher concentrations of Ab
 - Occurs 3-7 days after exposure

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Case Question 5

Co-stimulatory pair CD40/CD40L is important for both Th and B cell activation. From the statements below, please select the one that incorrectly describes the effect this pair-binding has on B cell.

- A. Promotes downregulation of MHC and costimulatory molecules
- B. Promotes somatic hypermutation/affinity maturation
- C. Required for Ab "class switch"
- D. Promotes memory B cell formation

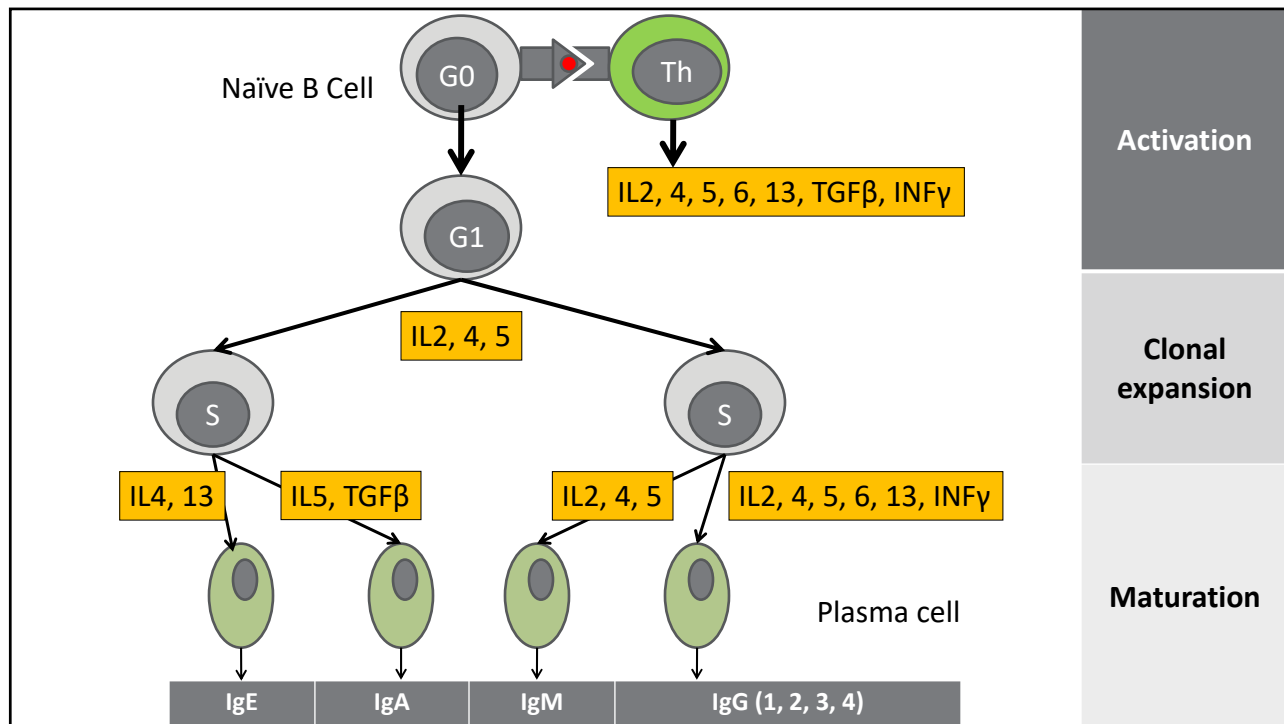
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Antibody Effector Phase			
Function	Antibody	Effect	Outcome
Neutralization	IgM, IgG, IgA	Block adhesion of viruses and attachment of toxins	Inactivates viruses and bacterial toxins
Agglutination	IgM, IgG, IgA	Cells clump together	Reduces number of infectious units to be dealt with
Complement activation	IgM, IgG	Ag-Ab complex activates classic complement pathway (IgG3 and IgG1 most efficient)	Inflammation and cell lysis
Ab dependent cell mediated cytotoxicity	IgM, IgG	Antibodies attach to cells and mark it for elimination	Destruction by non-specific binding of NK cells via surface Fc receptors
Ab dependent cell mediated phagocytosis	IgG	Coating cell with antibody (Fc receptor)	Enhanced phagocytosis by macrophages and neutrophils

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Antibody mediated rejection pathways

1. Complement-dependent cytotoxicity (IgG and IgM) → activation of Classical pathway results in membrane attack complex formation
 - 70-85% of AMR
 - Depends on donor specific Ab concentration and its ability to bind C1q
 2. Antibody-dependent cell-mediated cytotoxicity (IgG and IgM)
 - Natural killer cells have Fc receptors
 - Antibodies direct and activate cytotoxic cells via nonspecific Fc receptor binding
 3. Antibody-dependent cell-mediated phagocytosis (IgG)
 - Aggregation of antibodies on surface of pathogens → enhanced phagocytosis
- Ultimate result → endothelial damage and necrosis, loss of vascular integrity, increased coagulation → antibody mediated rejection

Womer, K., & Rabb, H. (2010). Immunologic Principles in Kidney Transplantation. In Comprehensive Clinical Nephrology (pp. 1119-1133).

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B Cell Decline Phase: Negative Regulation

- Down regulation of humoral immune response occurs via three mechanisms:
 - Antibody eliminates Ag source → no further stimulation
 - When enough soluble antibody-antigen complexes have been generated → inhibit B cell activation
 - Clustering of membrane Ig with Fc receptor on surface of B cell activates an inhibitory signaling cascade that terminates the B cell activity

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ADAPTIVE IMS MECHANISMS OF INJURY TO THE ALLOGRAFT

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Adaptive IMS: Allograft Recognition

DIRECT PATHWAY

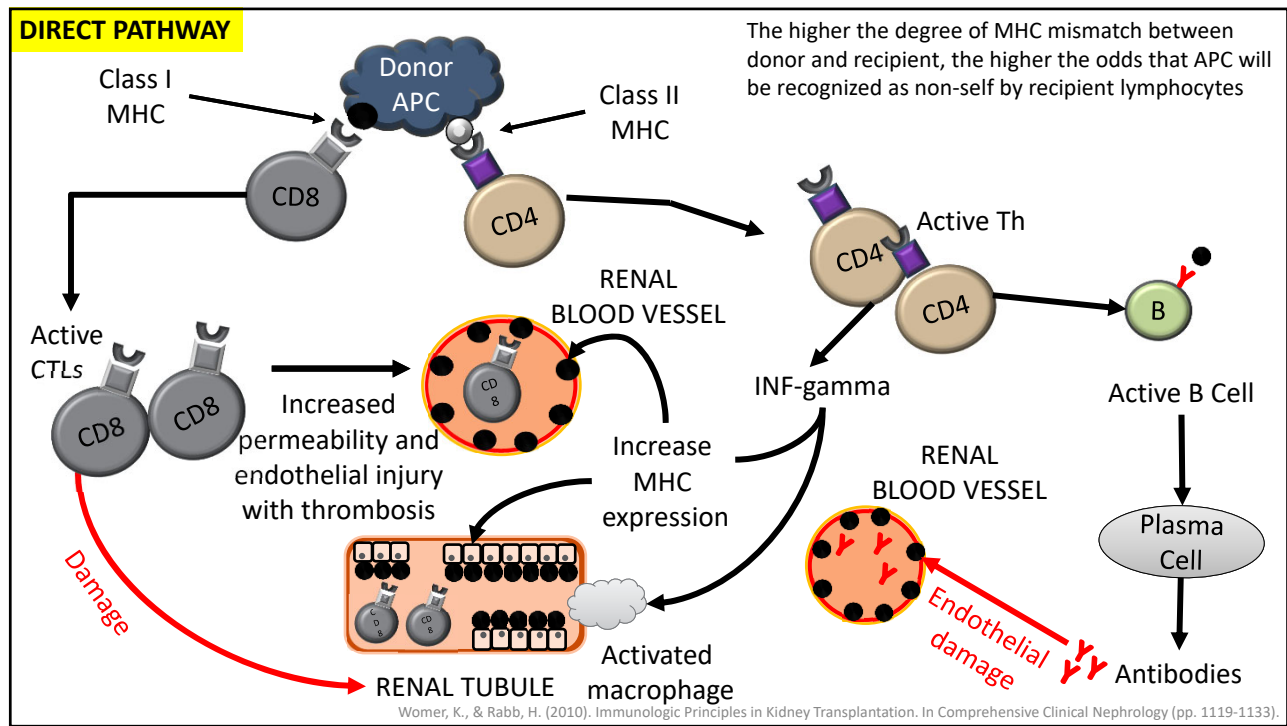
- Antigen presenting cell that activates T cells is donor derived
 - Dendritic cell>>>other
- Organs that are rich in lymphoid tissue inherently carry higher rejection risk

Small bowel/Lung>>
Heart/Pancreas/Kidney>>Liver

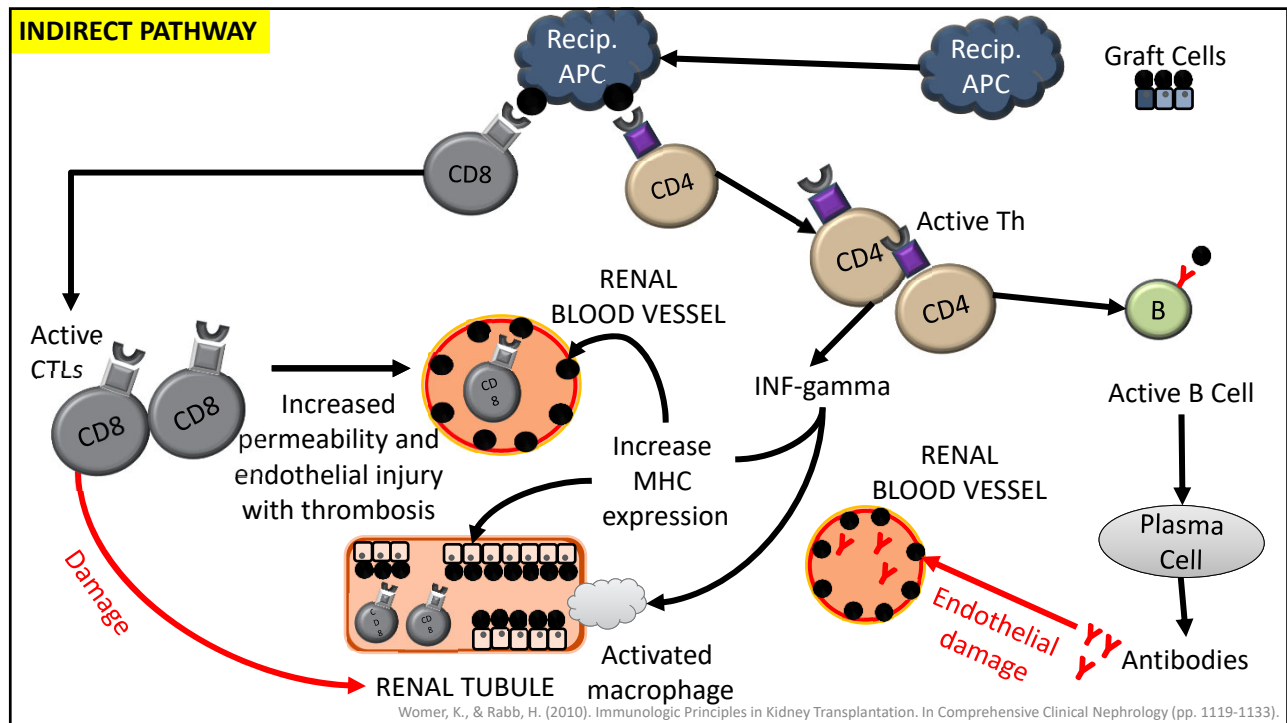
INDIRECT PATHWAY

- Antigen presenting cell that activates T cells is recipient derived
 - Dendritic cell, memory B cell, macrophage

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ESTIMATING REJECTION RISK

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Rejection Risk Depends On Previous Exposure To Foreign Antigen: Blood Group Ag

- Ab to blood group antigens are naturally occurring
- Blood group mismatch is an absolute contraindication for deceased donor transplants
 - Exceptions: A2/A2B donor subtype into B and O recipient
 - May be overcome in case of living kidney donor, and some living donor liver transplants

	Group A	Group B	Group AB "universal recipient"	Group O "universal donor"
Red blood cell type				
Ag present	A antigen 	B antigen 	A and B antigen 	None
Ab present	Anti-B	Anti-A	None	Anti-A and Anti-B

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Rejection Risk Depends On Previous Exposure To Foreign Antigen: HLA

- Ab to HLA are not naturally occurring
- Formed as a result of exposure to material containing non-self HLA
- Preformed donor-specific HLA Ab (DSA) are associated with graft failure
→ absolute contraindication for deceased donor transplants
- May be overcome in case of living renal donor and rarely deceased donor transplants


Blood
transfusion

Pregnancy

Previous organ or
tissue transplant

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Panel Reactive Antibody (PRA)

- Measures amount of pre-formed HLA Ab in potential recipient vs. random pool of HLA Ag from general population
 - Actual measurement vs. calculated PRA
 - Evaluated every 1-3 months as HLA Ab levels may fluctuate
 - Peak vs. current PRA is relevant due to immunologic memory
- Estimates rejection risk and predicts wait time
 - Patients with PRA more than 10-30% are often referred to as “sensitized”

Peak PRA (%)	Portion of waiting list	Median kidney waiting time
0-19	60%	490
20-79	21%	1042
>80	19%	2322

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Crossmatch (XM)

- It is a donor-specific HLA Ab (DSA) test that determines immunologic compatibility between the recipient and his or her identified donor
 - Measure of recipient serum reactivity to donor lymphocytes (T and B cells)
- Cell based assay:
 - Combine recipient serum (potentially containing DSA) with donor T and B lymphocytes (containing donor HLA antigens)

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Crossmatch (XM)

Complement Dependent Cytotoxicity (CDC-XM) or Standard XM

- Complement is added to the mixture of donor lymphocytes with recipient serum and detection of cell lysis is performed
- Qualitative test; positive or negative
 - Positive implies enough DSA to trigger complement cascade and cytotoxic response
 - Positive CDC XM is considered contraindication to transplantation
- Functional but lacks sensitivity and specificity

Flow (FXM)

- Flow cytometer analysis of DSA activity reported as intensity of fluorescence above control (Mean Channel Shifts)
- Quantitative; with lab specific positivity thresholds
 - Positive implies DSA-Ag binding, but we do not know which Ab or if it has complement-fixing ability
 - Living donor transplant may proceed if desensitization used
- Non-specific; Pronase, DDT improve specificity and sensitivity

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Crossmatch Interpretation

T-Cell XM	B-Cell XM	Interpretation
Neg	Neg	No DSA to HLA class I or II OR DSA titer too low to be detected
Pos	Pos	DSA to HLA class I only OR DSA to HLA class I and II
Neg	Pos	DSA to HLA class II OR Class I DSA titer too low to be detected
Pos	Neg	B cells likely not viable (repeat test) OR Error (repeat test)

- Recall: T cells only have MHC class I Ag on their surface, B cells express both MHC I and II Ag
 - Positive T cell XM implies presence of Class I Ab in which case B cell XM should also be positive
- Dilemma of weakly positive B-cell XM
 - Belief that Class II DSA are less significant in acute AMR → challenged by findings linking indolent Class II DSA with chronic AMR
- Dilemma of interfering autoantibodies
 - Use Dithiothreitol (DTT) treatment to disrupt IgM (autoAb are predominantly IgM)
 - Use auto-crossmatch

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Donor Specific Antibody Screen

- Solid phase assay using flow cytometer to detect specific HLA Ab
 - Quantifies how much DSA is present in recipient serum
 - Most sensitive and specific → single antigen bead assay
 - Beads coated with single recombinant HLA Ag
 - Bound antibody labeled with a fluorescent signal and detected using the Luminex fluoroanalyzer
 - May be augmented to identify DSA that can bind complement fraction C1q
- Reported as Mean Fluorescence intensity (MFI)
 - On average, strong MFI >5,000, moderate 2,000-5,000, weak 700-2000 and negative <700
- Virtual Crossmatch
 - Algorithm-driven analysis to determine if FXM would hypothetically be positive or negative
 - Based on data from PRA blood samples and solid phase assay used to determine and quantify DSA

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Other Reactive Allo-antibodies

- Present on endothelial cells
 - MHC class 1 related chain A and B (MICA and MICB) antigens
 - Anti-angiotensin-2 receptor
 - Anti-glutathione S-transferase T1
 - Anti-endothelial antibodies
 - Anti-endothelial antibody can be detected by using donor monocytes for crossmatch
- Generally, not screened for unless unexplained AMR

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Case Question 6

Kidney Transplant was performed using a kidney from a donor who was 3/6 antigen mismatch to the recipient. Patient was at a low risk for rejection (compatible blood group, 0% PRA, negative crossmatch and no preformed donor specific antibodies). Kidney function was normal for 3 years. However, 3-years post-transplant, patient was unable to afford his medications and stopped taking his immunosuppressants. Within two days of stopping his medications, the kidney was rejected. What type of rejection do you expect to find in this patient?

- A. Acute rejection due to cell-mediated immunity
- B. Hyperacute rejection
- C. Chronic rejection due to antibody mediated immunity
- D. Humoral rejection mediated by natural killer cells, mast cells and neutrophils

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TOLERANCE

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What Is Immunologic Tolerance?

- Is a state of unresponsiveness to substances or tissue that have the capacity to elicit an immune response
- Central Tolerance
 - T cell and B cell development events (negative and positive selection)
 - Restricted to maturation sites (thymus and bone marrow)
- Peripheral Tolerance
 - Mature T cell and B cell events in periphery
 - Restricted to spleen, lymph node, and non-lymphoid tissue

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Transplant Tolerance

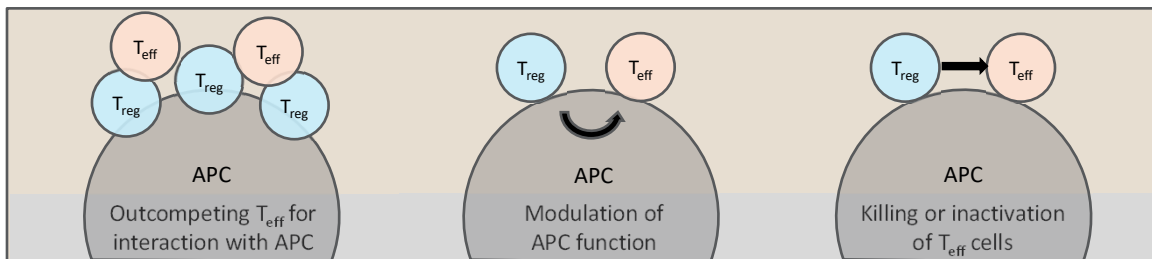
- State characterized by absence of destructive immune response in the recipient toward a well-functioning allograft, with a fully intact immune system and no exogenous immunosuppression
- Central tolerance to transplant – reprogramming IMS
 - Deletion – removal of the donor-reactive immune cells
 - Re-education in thymus via donor hematopoietic progenitor cells
 - Induction of chimerism
- Peripheral tolerance to transplant
 - Deletion
 - Induction of anergy (nonresponsiveness; by eliminating co-stimulatory interactions or pharmacologic manipulation of APCs)
 - Modulation of Treg activity

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Treg Mediated Suppression Mechanism

1. Activated T_{reg} out-compete Ag-specific effector T cells (T_{eff}) for MHCII binding because of high expression of adhesion molecules (LFA-1); also express high levels of inhibitory CTLA-4
2. Downregulate B7 expression on APC modulating APC function
3. May kill T_{eff} cells directly by secreting granzyme/perforin or inactivate T_{eff} by producing immunosuppressive cytokines (IL-10, TGF-beta)



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Inducible Treg Cells

- Inducible Treg are generated from Th0 cell in presence of immunomodulatory cytokines (TGF-beta, IFN-gamma, IL-10) or repetitive stimulation of APCs
- Inducible Treg suppression is NOT contact dependent
 - Suppressive function is not Ag specific
- Tasked with helping turn off immune response to foreign-Ag once response is over

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Can We Induce Tolerance In Mature T Cells?

- Central tolerance
 - Achievable during embryonic life where activation of adaptive IMS with a non-self Ag creates a chimera
 - Immunologically mature individual does not respond to that same non-self Ag at later exposure
 - Development in adults is more difficult – requires functioning thymus, high dose of Ag, prolonged period of exposure and intense immunomodulation
- Peripheral tolerance does not last indefinitely; with time it wanes and eventually disappears
 - Depends on persistence of tolerogen

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Is Accommodation Tolerance?

- Accommodation observed in ABO incompatible kidney transplants that function normally in recipients with high titers of anti-blood group Ab directed against antigens in the grafts
 - A donor into B recipient
- Phenomenon of “accommodation” is not an example of tolerance
 - Absence of immunity can be ascribed to tolerance (also immunosuppression)
 - Absence of injury in the face of immunity is typical of accommodation
- Accommodation differs from tolerance in that IMS retains ability to reject fresh tissue from the same donor, but accommodated donor tissue remains protected
 - Mechanism remains unclear

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Key Takeaways

- Innate immune system plays a key role in ischemia reperfusion injury, maintaining inflammation and facilitating adaptive immune response
- Cytokine IL2 is essential for full activation of adaptive immunity and modulation of IL2 secretion as well as receptor function results in inhibition of adaptive response
- Appropriately targeted Inhibition of classic complement pathway can result in inhibition of cell lysis and inflammation
- Alloantigen recognition is mediated by the adaptive immunity resulting in Tc cells eliminating antigen via cellular rejection, B cells via antibody mediated rejection and Th cells play essential role in propagating both pathways
- Stratifying rejection risk using PRA, crossmatching and DSA ensures appropriate organ allocation and minimizes rejection risk
- Developing tolerance to allograft is possible but requires intense immunomodulation and induction of regulatory T cells that is often difficult to achieve and maintain

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Transplant Immunology

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