T Cell Maturation,Activation and Differentiation

- **Positive Selection- In thymus, permits survival of only those T cells whose TCRs recognize self-MHC molecules (self-MHC restriction)**
- **Negative Selection- eliminates T cells that react too strongly with self-MHC or with self-MHC plus self-peptides.**

When arrive at thymus progenitor T cells do not express surface molecules (I.e., no CD4, 8 or TCR). Not yet re-arranged TCR genes

- **T progenitor cells enter cortex**
- **spend 3 weeks in development with changes in surface markers**
  - **double negative at first (no CD4 or CD8)**
  - **c-Kit (receptor for stem-cell growth factor), CD44 (adhesion molecule), and CD25 (the alpha chain of IL-2 receptor)**
  - **Cells continue to proliferate and DO NOT express TCR.**
  - **Cell stops proliferating, stops expressing c-Kit, reduce CD44 and begin to rearrange TCR genes**
  - **rearrange Beta TCR chain and this associates with a pre-T alpha chain (pre-T cell receptor [pre-TCR])- this recognizes some intrathymic ligand and transmits a signal through CD3 that activates Lck, a protein tyrosine kinase**

- **The activation of TK selects cells expressing beta chain for further expansion and maturation**
- **Suppresses further rearrangement of beta chain TCR gene**
- **Enhances rearrangement of alpha chain TCR**
- **Induces development to double positive $CD4^{+}8^{+}$**
- **These cells proliferate, but alpha cannot rearrange because RAG-2 is degraded quickly in cells that are proliferating. This creates greater diversity by generating a clone of cells with a single TCR beta chain rearrangement which can then associate with many different alpha chains**

- **As TCR alpha is being made the T cell begins undergoing positive and negative selection**
- **Double positive (DP) cells express the alpha and beta TCR-CD3 complex and develop into either single-positive $CD4^+$ or $CD8^+$ cells.**

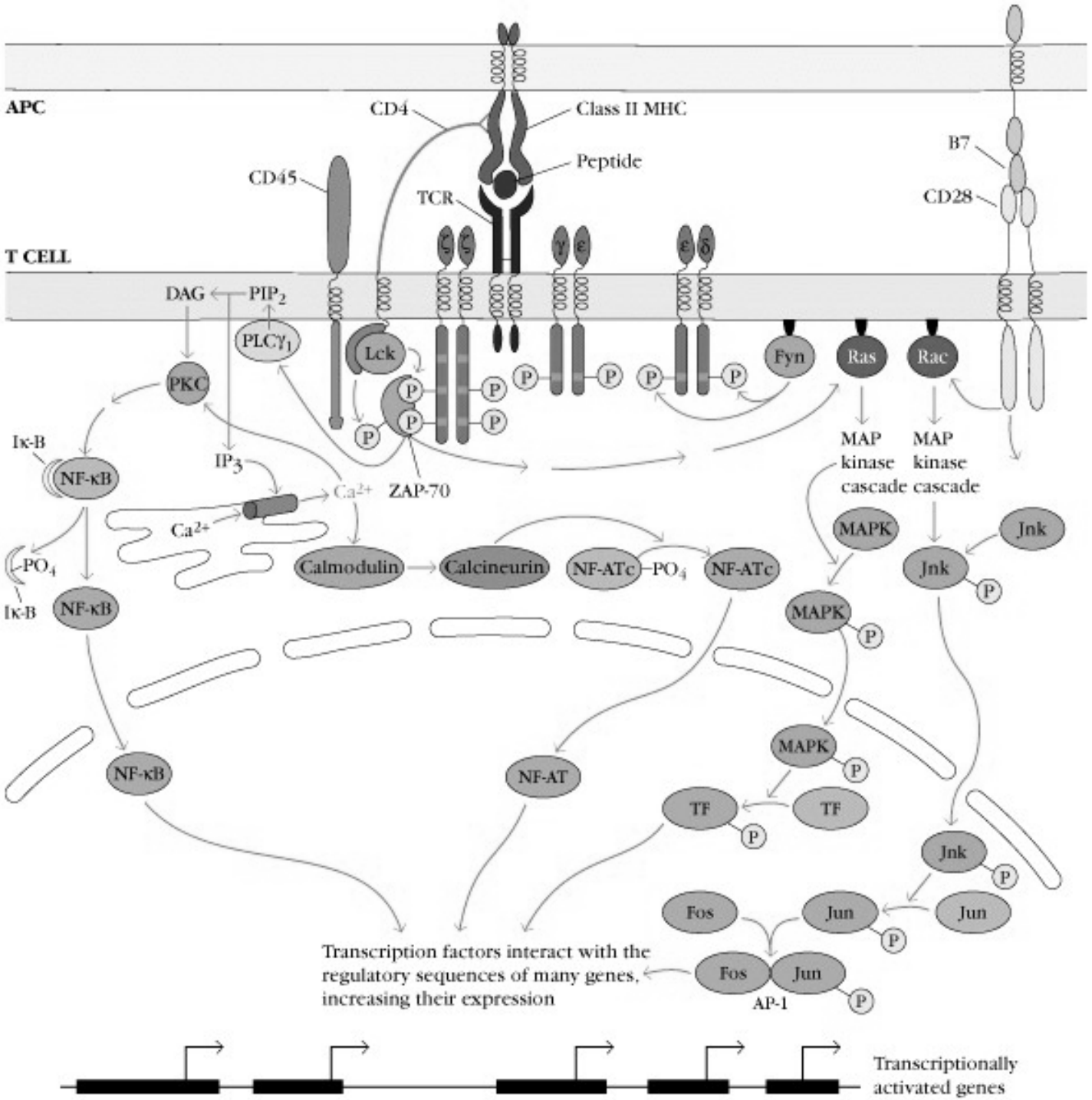
T_H Cell Activation

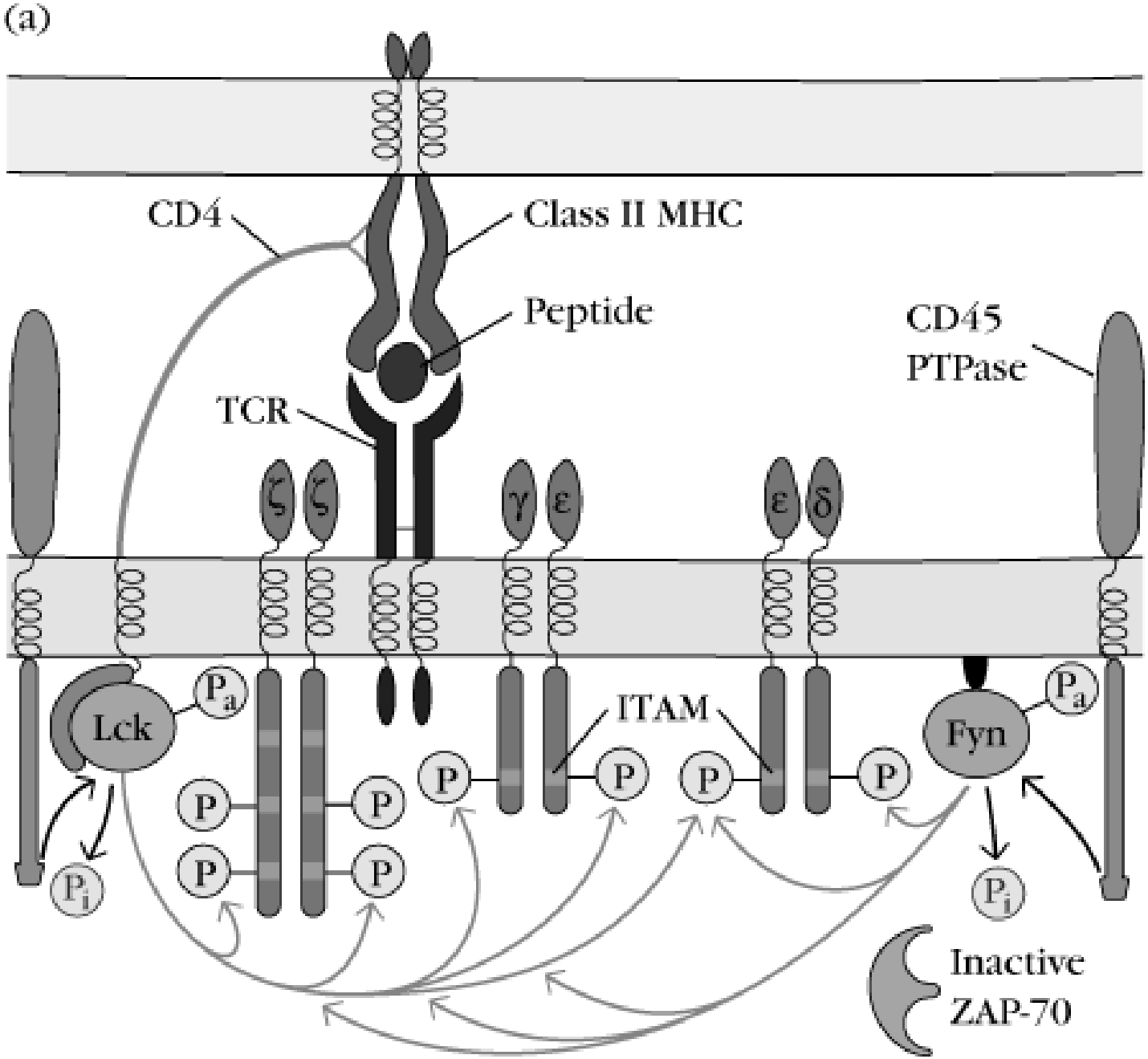
- **Initiated by interaction of TCR-CD3 complex with processed antigenic peptide bound to class II on APCs**
  - **proliferates into memory and effector cells, and many genes activated (see table 10-3).**
    - **Immediate genes- Fos, jun, NF-AT, myc, NFkB**
    - **early genes-- IFN-gamma, IL-2, IL-2R, IL-3, TGF, …..**
    - **Late genes- HLA-DR, VLA-4 and other VLAs, …. (adhesion molecules)**
  - **These events brought about by signal transduction:**
    - **receptor --> 2nd messengers (DAG, PI_3, cAMP, CA^{++})--> protein kinases (TK, PKC, calmodulin….)---> ---> gene activation through NF (nuclear binding factors)**

- **Cross-linking of TCR necessary & occurs by linking with MHC-peptide complexes (can activate using F(ab)'$_2$ or antibody that cross links CD3.**
- **Activation of ITAM (immunoreceptor tyrosine-based activation motif) on cytoplasmic tail of CD3 molecules**
  - **Phosphorylation of tyrosines in ITAMs by *Fyn* and *Lck* is early consequence of cross-linking TCR. Phosphorylation of inhibitory site (turns off activity) and active site. To be active not only must be phosphorylated at active site but inhibitory P must be removed by phosphatase (CD45- transmembrane phosphatase)**

- **Once released the active *Lck* and *Fyn* phosphorylate tyrosine residues in ITAMs of CD3 complex**
  - **phosphorylation of ζ Chain creates a docking site for ZAP-70 (zeta-associated protein) and when this binds to ITAM the TK activity of ZAP-70 is activated by *Lck* and *Fyn* phosphorylation. Activation of ZAP-70 leads to activation of many pathways (I.e., PKC (activates and causes release of NF- κB) [IP_3 and DAG], calcineurin (calmodulin-dependent phosphatase) which dephosphorylates the inactive cytosolic form of the nuclear factor NF-AT.**
  - **Both NF factors activate genes (I.e., IL-2 and IL-2R)**

- **T cells require two signals for activation**
  - **Signal 1: interaction of an antigenic peptide with the TCR-CD3 complex**
  - **Signal 2: Antigen-nonspecific co-stimulatory signal provided by interactions between CD28 on the T cell and B7 proteins on APC.**
    - **Ligands for B7 are CD28 and CTLA-4 (CD 152) [these act antagonistically with each other]**
    - **CD28 expressed on both resting and activated T cells but CTLA-4 found only on activated cells (24 h after stimulation).**
    - **CD27 & B7 stimulates (augments) IL-2 production and proliferation.**
  - **Signal 1 without signal 2 produces ANERGY**


APC
T CELL
CD4
Class II MHC
Peptide
TCR
CD45
B7
CD28
DAG
PIP2
PLCγ1
Lck
Fyn
Ras
Rac
PKC
ZAP-70
Iκ-B
NF-κB
IP3
Ca2+
MAP kinase cascade
MAP kinase cascade
MAPK
Jnk
Calmodulin
Calcineurin
NF-ATc
PO4
NF-AT
TF
Fos
Jun
AP-1
Transcription factors interact with the regulatory sequences of many genes, increasing their expression
Transcriptionally activated genes


(a)
CD4
Class II MHC
Peptide
CD45
PTPase
TCR
ζ
ζ
γ
ε
ε
δ
Lck
Pa
ITAM
Fyn
Pa
P
P
P
P
P
P
P
P
Pi
Pi
Inactive
ZAP-70

- **Superantigens- Bind simultaneously to the V_β domain of a T-cell receptor and to the α chain of a class II MHC molecule. (outside of TCR cleft)**
  - **Exogenous superantigens-- soluble proteins secreted by bacteria (I.e., staphylcoccal enterotoxins, toxic shock syndrome toxin, exfoliative-dermatitis toxin, mycoplasma-arthritidis supernatant and streptococcal pyrogenic exotoxins.**
  - **Endogenous superantigens-- cell-membrane protein encoded by certain viruses that infect mammalian cells. These viral proteins are called minor lymphocyte stimulating (Mls) determinants.**
- **Since superantigens bind outside of the TCR antigen-binding cleft any T cell expressing a particular V_β sequence will be activated (polyclonal response).**

- **T-cell differentiation-**
  - **CD4+ and CD8+ cells leave thymus and enter circulation as resting cells (naïve cells). These continually recirculate between blood and lymph system. During recirculation the naïve T cells reside in the LN and Spleen, but if it does not encounter antigen it exits and rejoins blood. Circulates from blood to LN and back to blood every 12-24 hours.**
  - **1 in 10^5 naïve T cells specific for any given antigen, and recirculation increases chances that it will encounter antigen**
  - **Thought to survive only 5-7 weeks if it does not encounter Ag. Some cells may life a lot longer**

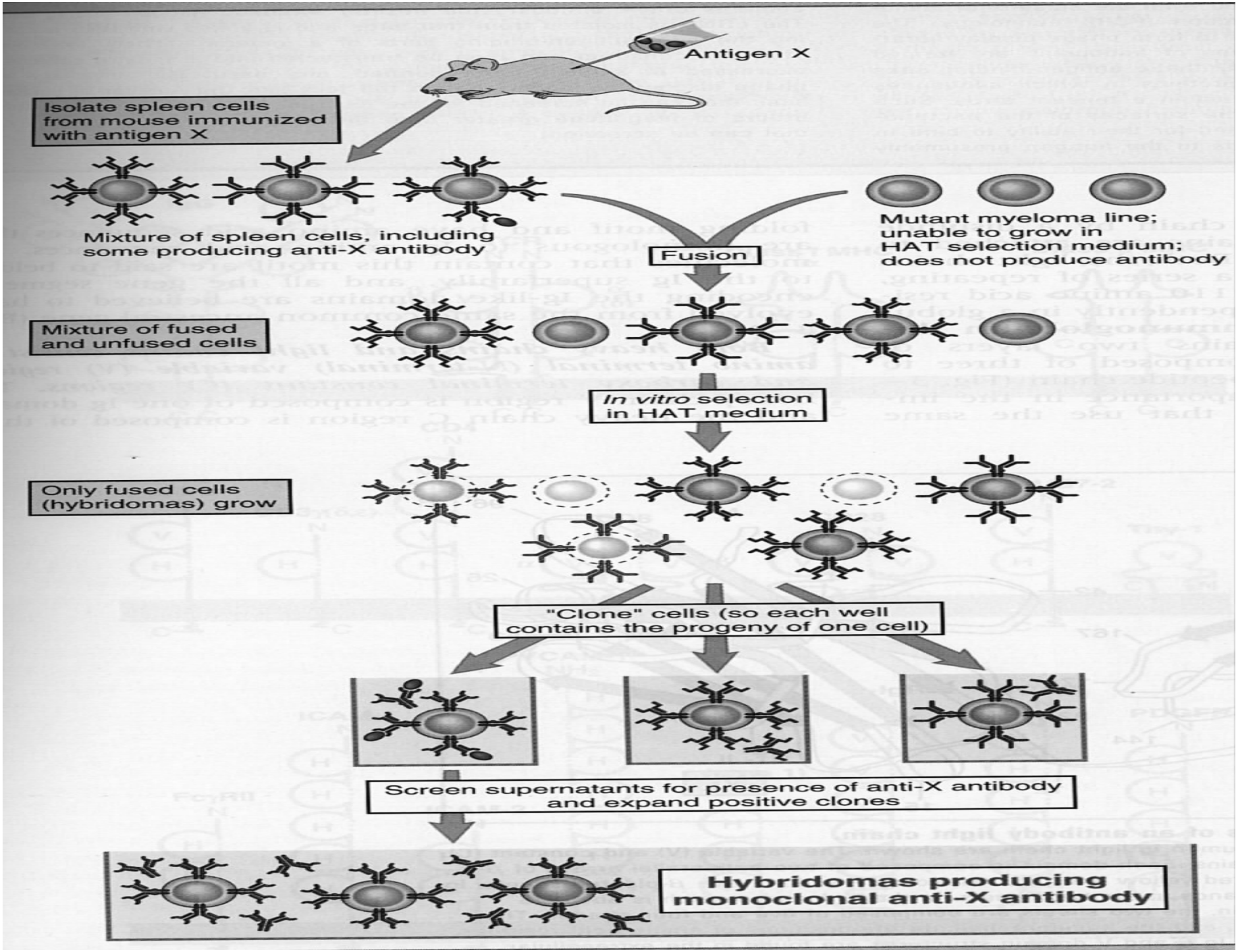
- **Effector & Memory T cells-**
  - **After Ag encounter, naïve T cell enlarges into blast cell (~48 hours) and proliferates. At same time stabilization of IL-2 mRNA increases production by 100X, and secretion causes it to bind to IL-2R (divides 2-3 times for 4-5 day period). Produces large CLONE which differentiate into memory and effector cells.**

- **Effector T cells carry out specialized functions (cytokine production and B-cell help). These come from both naïve and memory cells and effector cells live short llife span (few days to a few weeks)**

- **2 populations of effector cells**
  - **T_H1 subset- secrete IL-s, IFN-γ , TNF-β . This subset is responsible for the classic cell-mediated functions (delayed type hypersensitivity and activation of T_C.**
  - **T_H2 subset- secretes IL-4, IL-5, IL-6, and IL-10. Functions more effectively as a helper for B-cell activation and class**
  - **switch reactions of Ig.**

Hybridoma Technology

- **Production of monoclonal antibodies**
  - **Antibodies that are from a single B cell (clone)**
  - **How to isolate and make**
    - **Fusion of B cell that produces antibody with a tumor cell that is immortal**
    - **Selection in HAT media**
      - **Unfused B cells will die because of limited life-span and tumor cells will die because they do not have the ability (due to mutation) to make their own purine bases (HAT is selective)**
  - **Select for cell that is producing antibody you want**


Antigen X
Isolate spleen cells from mouse immunized with antigen X
Mixture of spleen cells, including some producing anti-X antibody
Mutant myeloma line; unable to grow in HAT selection medium; does not produce antibody
Fusion
Mixture of fused and unfused cells
In vitro selection in HAT medium
Only fused cells (hybridomas) grow
"Clone" cells (so each well contains the progeny of one cell)
Screen supernatants for presence of anti-X antibody and expand positive clones
Hybridomas producing monoclonal anti-X antibody