# Tumor Immunology

- Does it exist?

  i.e., does the immune system recognize and eradicate cancer cells? Is there any evidence for immunological surveillance (Burnett and Thomas)?
- How can the immune system recognize cancer if it is essentially self-tissue? (Tolerance)
- If it does not- can it be made to do so?

  (Immunization designed to Break Tolerance)

Where is the danger-the innate activator?

# The Good News/Bad News Story

The immune system can destroy self-tissue quite effectively in autoimmunity, and in a tissue-specific (antigen-specific) manner: (thyroiditis, hepatitis, pancreatitis (diabetes), vitiligo, ITP, AIHA, gradt rejection etc.). So, self-tissue destruction can be potent.

- Are there ongoing anti-tumor immune responses in patients with cancer?
  - Spontaneous remissions are rare but can occur, renal cell CA, melanoma, and are associated with anti-tumor Abs and CTLs.

TIL cells (tumor infiltrating cells) include CTLs that recognize melanoma antigens/peptides (6/11 patients). But these CTLs were anergic:could not kill targets or produce γ-IFN. Many patients make anti-tumor antibodies, but are mostly IgM-will not efficiently induce effector responses-and may indicate a lack of T cell priming.

- So..the good news is that immune recognition of tumor antigens occurs but the bad news is that this occurs without activation of immune effector responses.

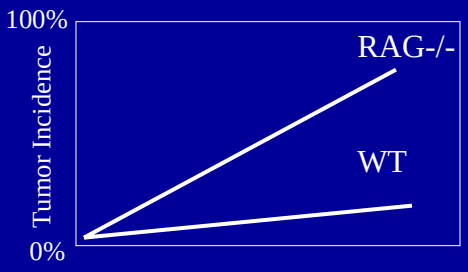
## More "good" news

### Evidence for Immunological Surveillance in Man

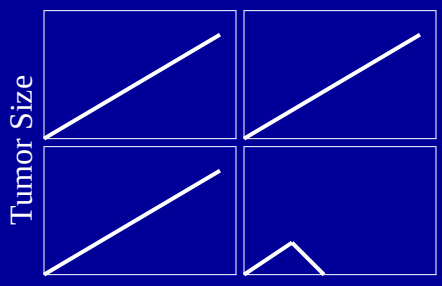
### Cancer Incidence Increases in Immunosuppressed

- Increased incidence of malignancies in HIV patients: EBV lymphoma, KS, squamous cell CA –but many of these are virally induced malignancies; this merely shows that eliminating a T cell response against **viral** antigens allows for the outgrowth of virally-transformed cells. Common variety neoplasms (colon, breast, prostate, lung, etc.,) are not increased.
- In transplant associated EBV lymphomas (presumably arise after the loss of EBV specific CTLs associated with T-cell depleted allo-BMT. Cures are achievable by infusion of donor T cells (reconstitute CTL response). Again loss of an anti-viral responses is implicated. (post-transplant patients are also at increased risk for melanoma and sarcoma).

**Immunosurveillance:** Tumors which Evolve in Lymphocyte Deficient Hosts are Rejected in WT Mice




Tumor (Sarcoma) Incidence is Increased in MCA-treated Lymphocyte Deficient Mice

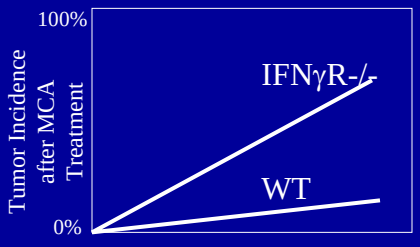


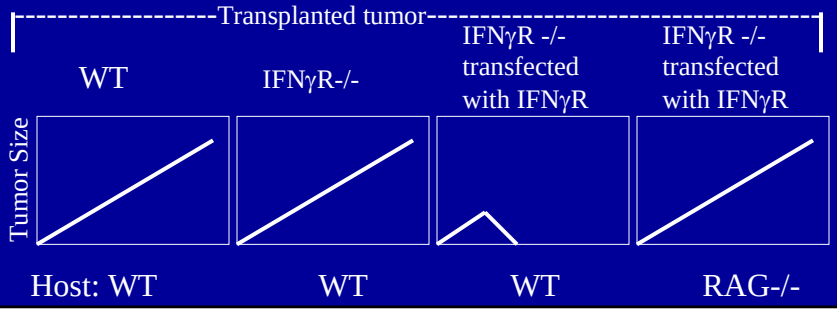

Tumor: WT origin

RAG-/- origin

Tumors which developed in RAG-/- hosts are REJECTED in WT Recipients

**Immune Surveillance**: Tumor Cell Expression of IFNγ Receptor is Required for Lymphocyte-Mediated Tumor Rejection



Immune surveillance:

1. Innate system

NK, NKT, gamma/delta T cells

↓↓↓

IFN-γ

↓

IL-12 (APC)

↓

2. Functional conventional T cells

**Table 1. Enhanced susceptibility of immunodeficient mice to formation of chemically induced and spontaneous tumors**

| Phenotype or depletion | Immunodeficiency | Tumor susceptibility |
|---|---|---|
| RAG-2$^{-/-}$ | T, B and NKT cells | MCA-induced sarcomas[49]<br>Spontaneous intestinal neoplasia[49] |
| RAG-2$^{-/-}$ × STAT1$^{-/-}$ (RkSk) | T, B and NKT cells<br>Insensitive to IFN-γ and IFN-α/β | MCA-induced sarcomas[43]<br>Spontaneous intestinal and mammary neoplasia[43] |
| BALB/c SCID | T, B and NKT cells | MCA-induced sarcomas[100] |
| Perforin$^{-/-}$ | Lack of perforin | MCA-induced sarcomas[42,43,46]<br>Spontaneous disseminated lymphomas[42,47] |
| TCR Jα281$^{-/-}$ | Subset of NKT cells | MCA-induced sarcomas[42,44,100] |
| Anti-asialo-GM1 antibody | NK cells and activated macrophages | MCA-induced sarcomas[100] |
| Anti-NK1.1 antibody | NK and NKT cells | MCA-induced sarcomas[46,100] |
| Anti-Thy1 antibody | T cells | MCA-induced sarcomas[46,100] |
| αβ T cell$^{-/-}$ | αβ T cells | MCA-induced sarcomas[50] |
| γδ T cell$^{-/-}$ | γδ T cells | MCA-induced sarcomas[50]<br>DMBA/TPA-induced skin tumors[50] |
| STAT1$^{-/-}$ | Insensitive to IFN-γ and IFN-α/β | MCA-induced sarcomas[41,49]<br>Wider tumor spectrum in STAT1$^{-/-}$ × p53$^{-/-}$ (ref. 41) |
| IFNGR1 receptor$^{-/-}$ | Insensitive to IFN-γ | MCA-induced sarcomas[41,49]<br>Wider tumor spectrum in IFN-γ receptor$^{-/-}$ × p53$^{-/-}$ (ref. 41) |
| IFN-γ$^{-/-}$ | Lack of IFN-γ | MCA-induced sarcomas[42]<br>C57BL/6: Spontaneous disseminated lymphomas[43]<br>BALB/c: Spontaneous lung adenocarcinoma[43] |
| Perforin$^{-/-}$ × IFN-γ$^{-/-}$ | Lack of perforin and IFN-γ | MCA-induced sarcomas[42]<br>Spontaneous disseminated lymphomas[43] |
| IL-12$^{-/-}$ | Lack of IL-12 | MCA-induced sarcomas[44] |
| WT + IL-12 | Exogenous IL-12 | Lower incidence of MCA-induced sarcomas[101] |

Methylcholanthrene-treated wild-type (WT) mice were treated with IL-12 during tumor formation.

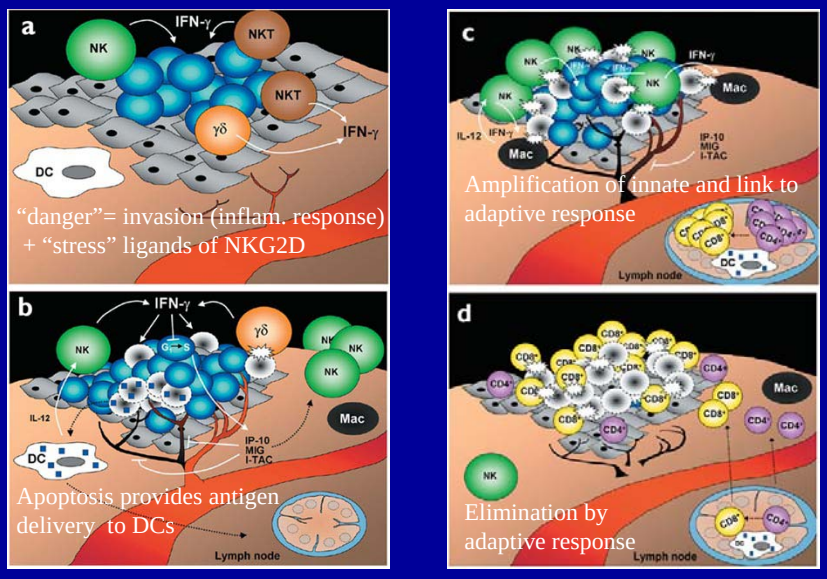
## More good news/ Evidence for Immunological Surveillance

- In mice, absence of IFN-γR, STAT1, IL-12, perforin, RAG, NK cells: All of these genetic deficiencies have an increased incidence of MCA (carcinogen) induced malignancies.

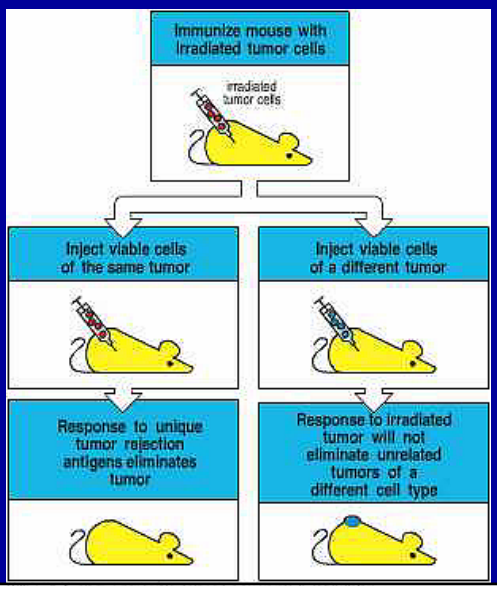
Evidence that IFN-induced antigen presentation by tumor cells provides immunity (as with viral immunity). IFN-γR -/- tumors grow in WT mice, unless transfected with TAP. Highly immunogenic tumors emerge in RAG -/- mice; these tumors grow in RAG -/- (in absence of immune selective pressure) but are rejected in WT mice (in presence of normal immune response).

Macrophages are primary source of IL-12 which induce NK and T cell production of IFN-γ. (activates STAT1)

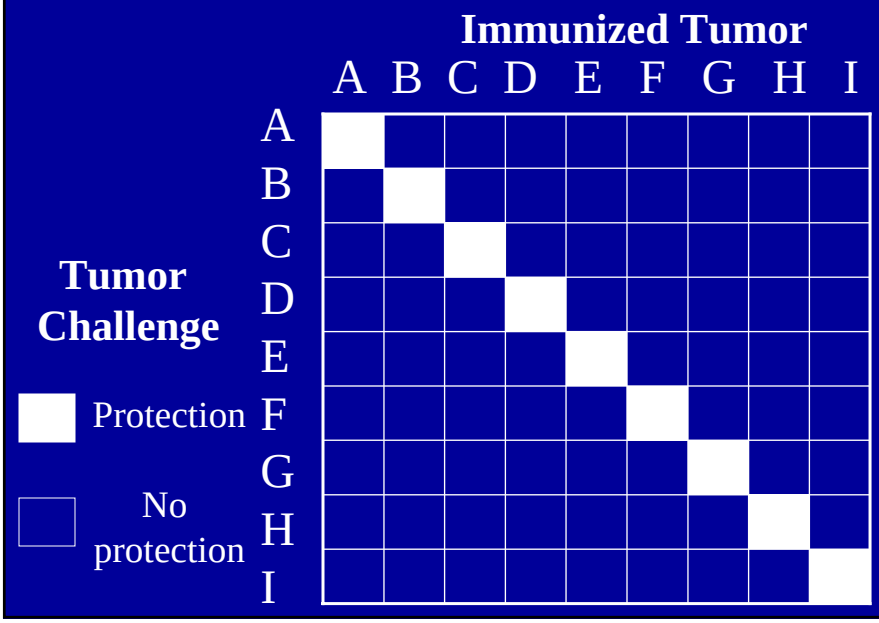
## Model of Innate Recognition and Initiation of the Adaptive Antitumor Immune Response




## Immunization with Tumor Cells Can Induce Protective Immune Response



## Tumor Antigens Are Unique to Individual Tumors




## Candidate Tumor Antigens

| Antigen Class | Antigen | Advantages/ Disadvantages |
|---|---|---|
| Whole Cell | Protein lysate or tumor RNA based expression | Universal<br>(Autoimmunity may be a problem) |
| Antigen-Specific | Peptide, DNA or recombinant protein | "Customized" therapy are required for these approaches. For whole proteins "antigen profile" of each tumor is required. Peptides require additional info. of indiv. HLA-type.<br>Antigenic modulation or loss (overcome by attacking multiple targets and antigens required for transformed phenotype). |

## Candidate Tumor Antigens..many more to come through genomics

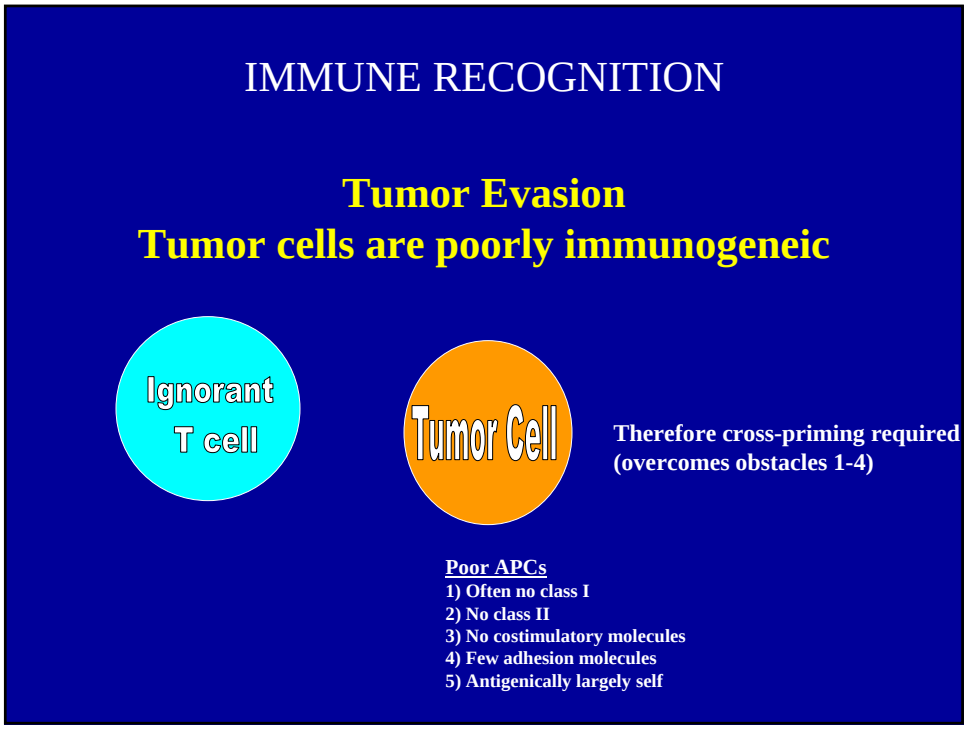
- **Shared Tumor Antigens** (common across tumors and tumor types) Allows single therapy to be applicable for many patients

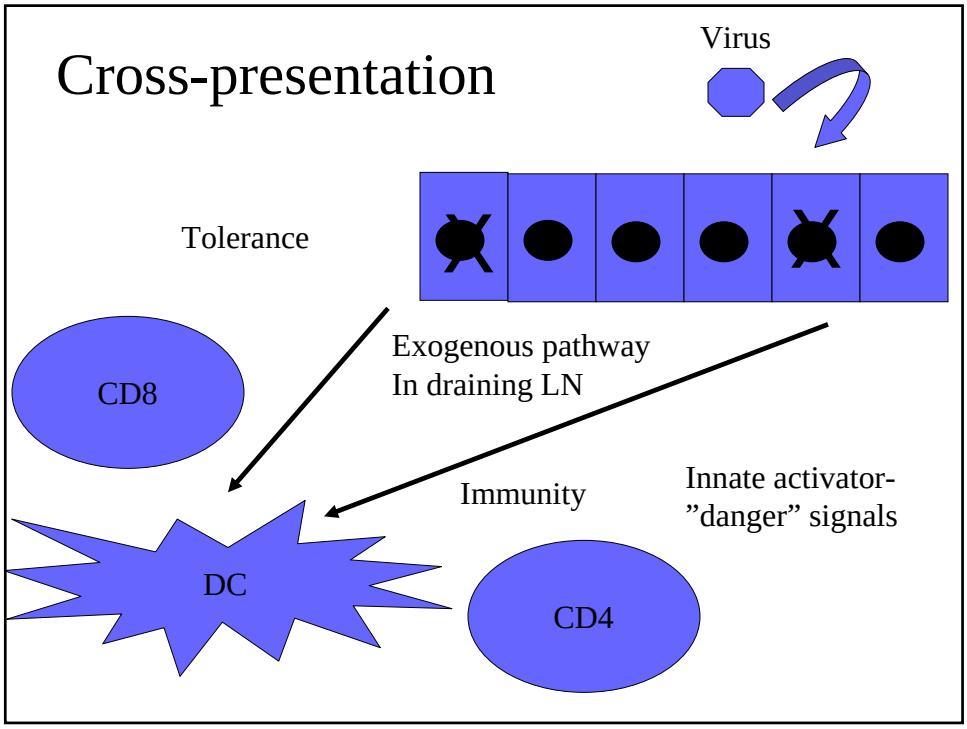
1. Cancer/testes genes
2. Differentiation associated antigens
3. Others including gangliosides, MUC-1, etc.,

- **Unique Tumor Antigens** (requires tumor specific therapy) Antigenic modulation would potentially interfere with malignant phenotype.

1. Overexpressed proto-oncogenes: EGFR, HER2
2. Point mutations: ras, β-catenin, CDC27, CDK4, Bcr/Abl
3. Viral Antigens: Human papilloma virus, EBV, Hepatitis B

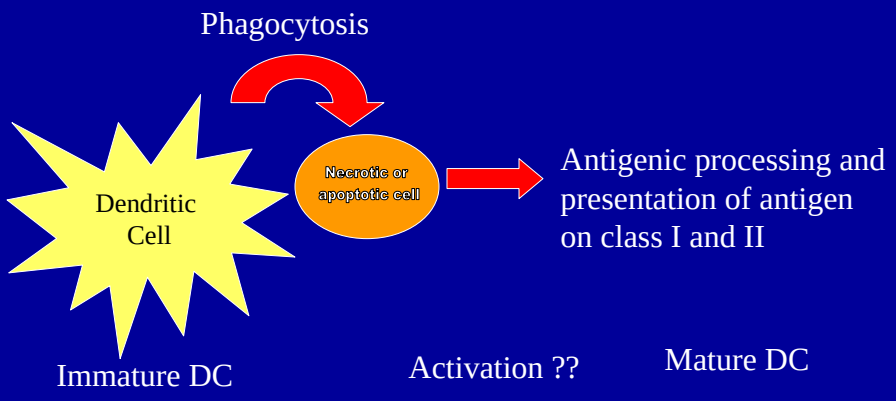
| Antigen Class | Antigen | Malignancy |
|---|---|---|
| Tumor Specific Antigen | Immunoglobulin Idiotype<br>TCR<br>Mutant ras<br><br>Mutant p53<br>p21-/bcr-abl fusion | B lymphoma, MM<br>T cell lymphoma<br>Colorectal, lung, bladder, Head and neck cancer<br>Pancreatic, Colon, Lung<br>CML, ALL |
| Developmental Antigens (cancer/testes genes) | MAGE-1, MAGE-3, GAGE family, 20 genes on the X chromosome<br>Telomerase | Melanoma but also in colorectal, lung, gastric<br>Various |
| Viral Antigens | Human Papilloma Virus<br>EBV | Cervical, penile cancer<br>Burkitt's lymphoma, nasopharyngeal Ca, post-Tx lymphoproliferative |
| Tissue-specific self-antigens (Differentiation antigens) | Tyrosinase, gp100,trp-1, trp-2<br>Prostatic acid phosphatase, PSA<br>Thyroglobulin<br>α-Fetoprotein | Melanoma<br>Prostate<br>Thyroid<br>Liver Cancer |
| Over-expressed self-antigens | Her-2/neu<br>CEA<br>Muc-1 | Breast and lung cancer<br>Colorectal, lung, breast<br>Colorectal, pancreatic, ovarian, lung |


IMMUNE RECOGNITION
Tumor Evasion
Tumor cells are poorly immunogeneic
Ignorant T cell
Tumor Cell
Therefore cross-priming required (overcomes obstacles 1-4)
Poor APCs
1) Often no class I
2) No class II
3) No costimulatory molecules
4) Few adhesion molecules
5) Antigenically largely self


Cross-presentation
Virus
Tolerance
Exogenous pathway
In draining LN
CD8
Immunity
Innate activator-
"danger" signals
DC
CD4

## IMMUNE RECOGNITION
## Cross-Priming

- Host somatic cellular antigens (i.e.not soluble antigens)are able to be presented to immune system by host APCs.
- True for viral antigens and cancer antigens.

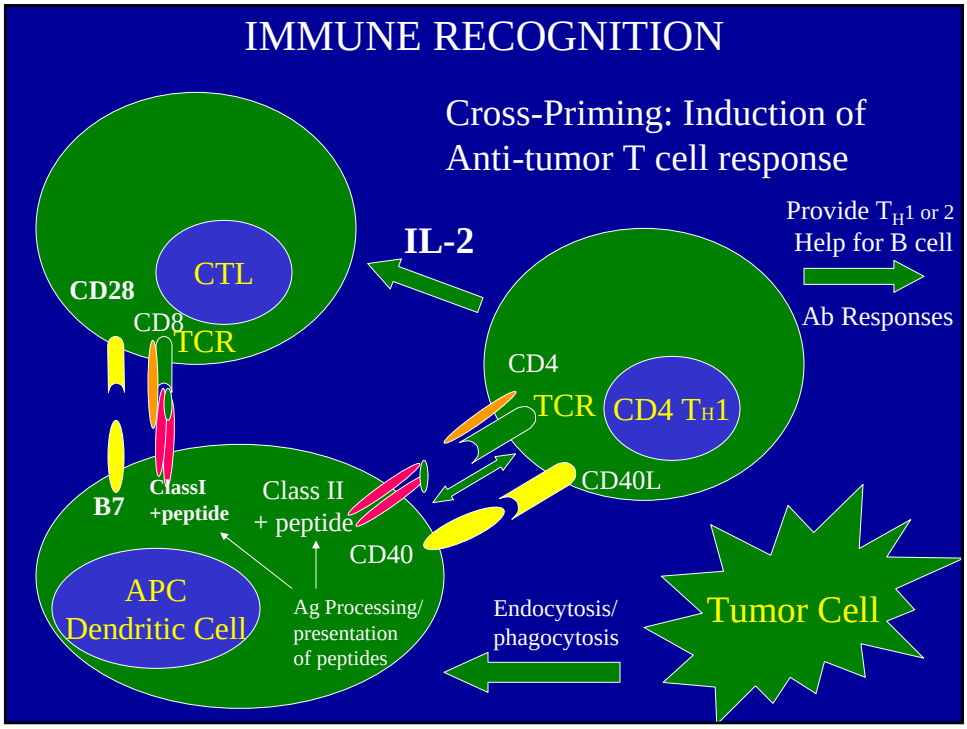


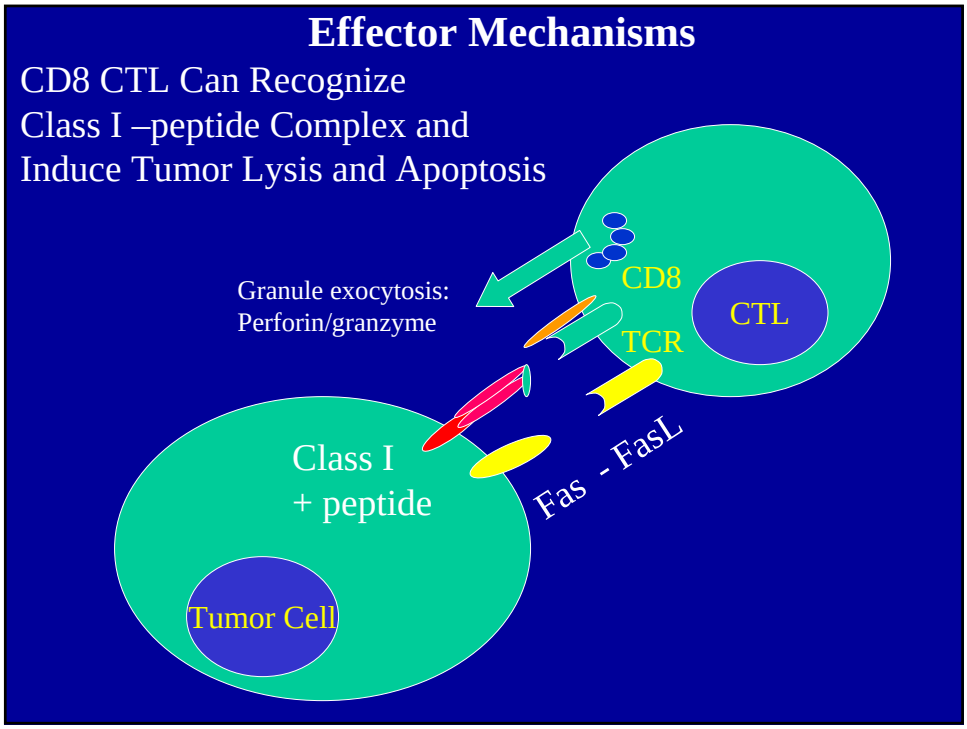

## Maturation Factors

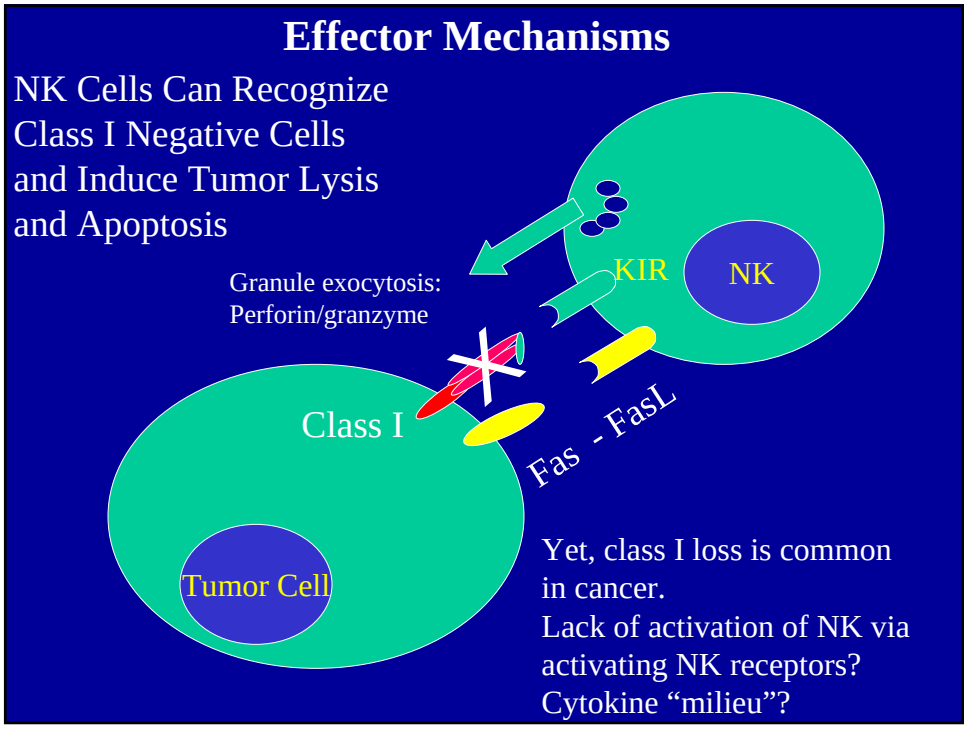
- T cell signals (encounter with specific Memory CD4 cell): **CD40L**
- **Microbial stimuli:** TLR ligands: LPS, hypomethylated DNA (CpG), dsRNA (poly dI:dC), peptidoglycans,
- Inflammatory Cytokines: TNF, IFN, (products of either Mϕ, NK or T cells)

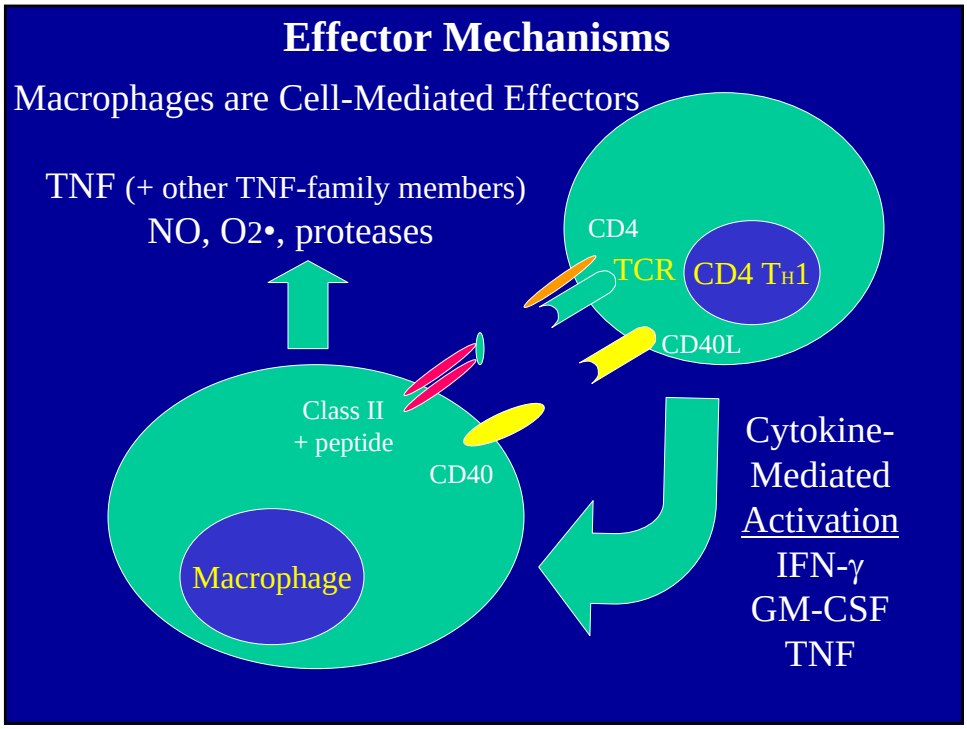
## Effective antigen presentation by "cross-priming" enhanced by DC activation/maturation (CD40L, TNF, others)

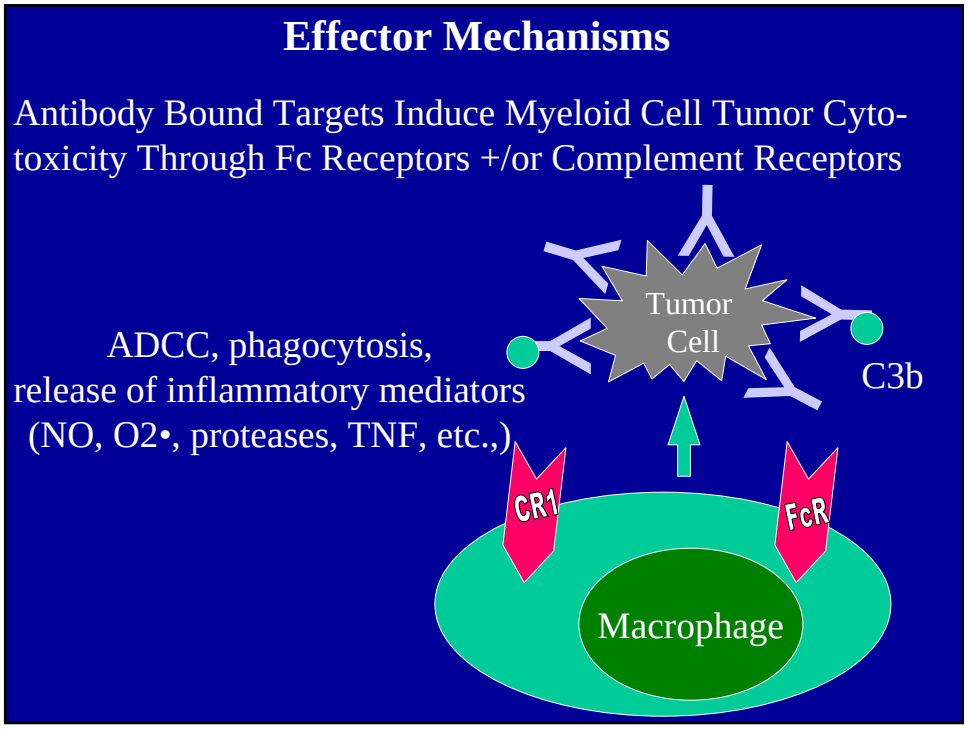
- Peripheral immature DCs migrate to LN upon activation by antigen/cytokines where they may encounter T cells.
- Maturation marked by transition of highly phagocytic/endocytic cell to a poorly phagocytic/endocytic cell.
- Upregulation of antigen processing and surface expression of class I and II molecules
- Upregulation of co-stimulatory molecules CD40, B7 (CD80,86) and adhesion molecules (ICAM-1) for interaction and activation of antigen-specific T cells.

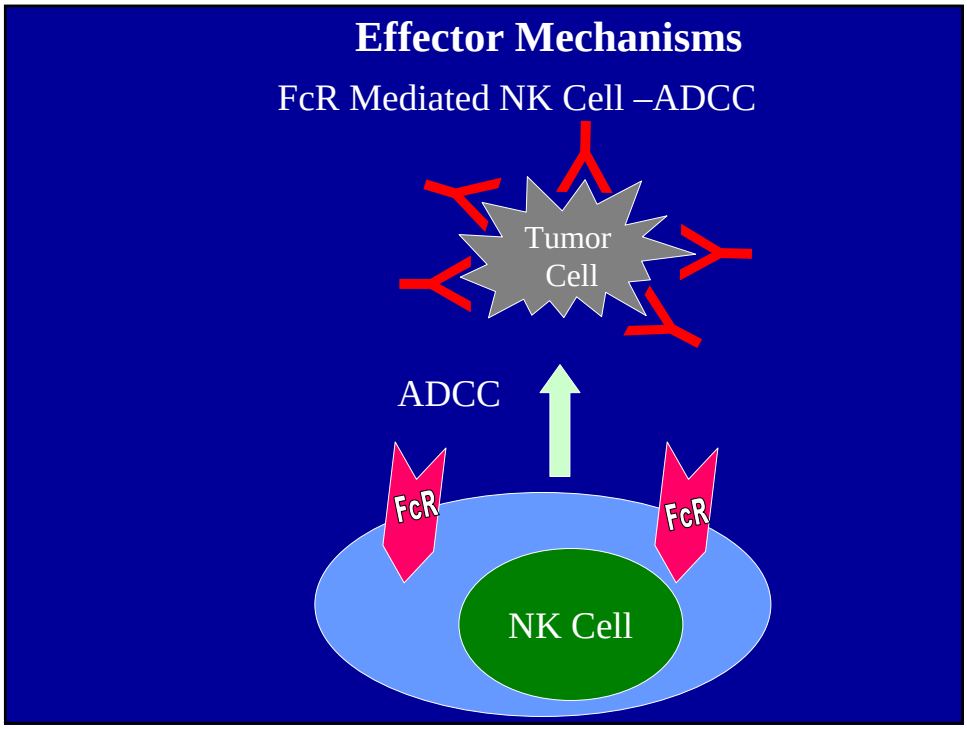


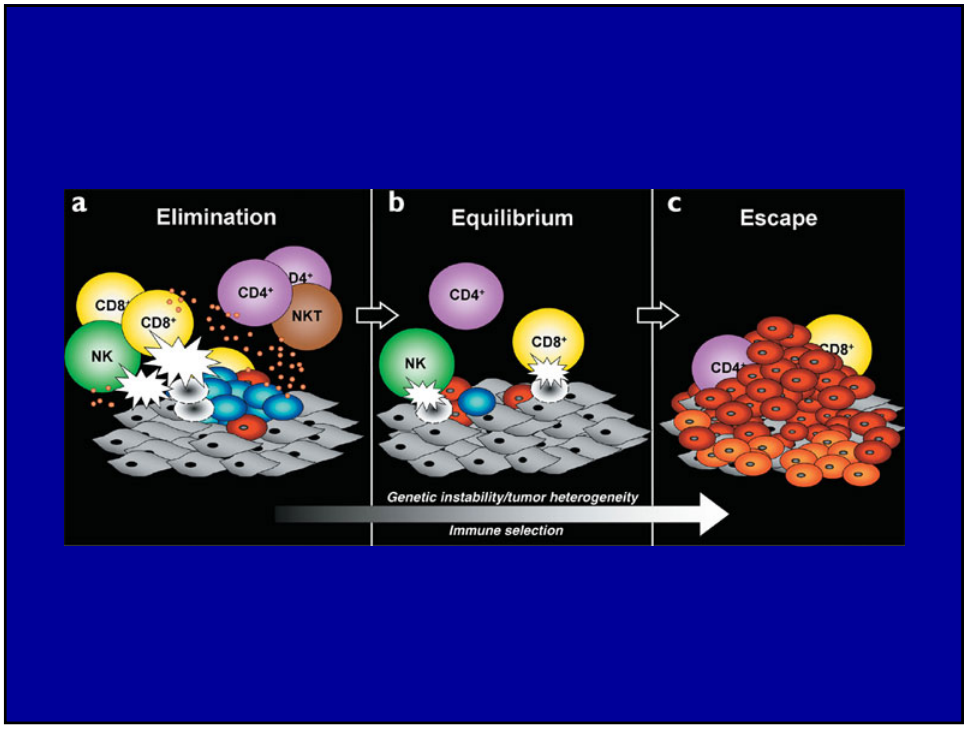

Effector Mechanisms
CD8 CTL Can Recognize
Class I –peptide Complex and
Induce Tumor Lysis and Apoptosis
Granule exocytosis:
Perforin/granzyme
CD8
CTL
TCR
Fas - FasL
Class I
+ peptide
Tumor Cell


Effector Mechanisms
NK Cells Can Recognize
Class I Negative Cells
and Induce Tumor Lysis
and Apoptosis
Granule exocytosis:
Perforin/granzyme
KIR
NK
Fas - FasL
Class I
Tumor Cell
Yet, class I loss is common
in cancer.
Lack of activation of NK via
activating NK receptors?
Cytokine “milieu”?


Effector Mechanisms
Macrophages are Cell-Mediated Effectors
TNF (+ other TNF-family members)
NO, O2•, proteases
CD4
TCR
CD4 TH1
CD40L
Class II
+ peptide
CD40
Macrophage
Cytokine-
Mediated
Activation
IFN-γ
GM-CSF
TNF


Effector Mechanisms
Antibody Bound Targets Induce Myeloid Cell Tumor Cyto-
toxicity Through Fc Receptors +/or Complement Receptors
ADCC, phagocytosis,
release of inflammatory mediators
(NO, O2•, proteases, TNF, etc.,)
Tumor
Cell
C3b
CR1
FcR
Macrophage


Effector Mechanisms
FcR Mediated NK Cell –ADCC
Tumor Cell
ADCC
FcR
FcR
NK Cell


a
Elimination
b
Equilibrium
c
Escape
CD8+
CD4+
NKT
NK
Genetic instability/tumor heterogeneity
Immune selection

## Tumor Evasion: Two separate problems

- Tumor antigens are not recognized by immune response-poorly immunogenic

(Immunologically *ignorant).*

- Tumors are resistant to or inhibit immune cytotoxic responses.

(active *suppression*—either dampen "priming" or avoid/inhibit/resist effector cell function).

## Bad News/Tumor Evasion Resistance to Effector Response

- Access to tumors may be limited by poor vascularity.
- Intrinsic resistance (anti-apoptotic genes).

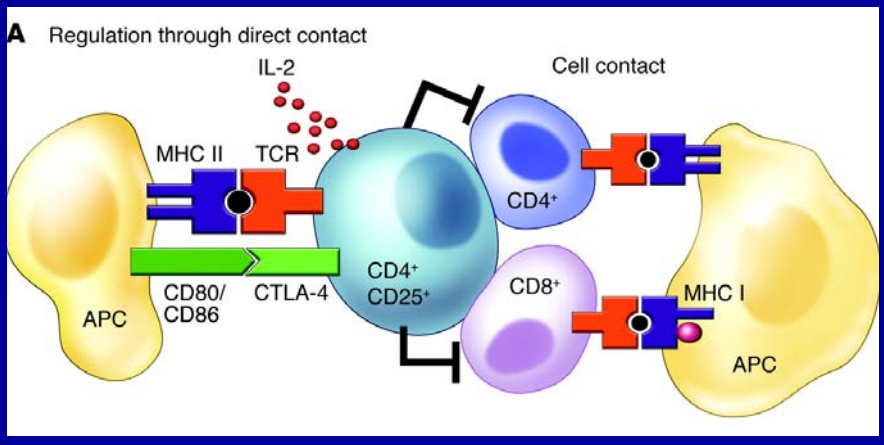
Resistance to death receptor pathways: Reduction of Fas receptor or enhanced expression of c-FLIP by tumors may render tumors resistance to fas-mediated apoptosis. Similarly, tumors commonly lose TRAIL receptors or express "decoy" receptors.

Upregulation of "survival" pathways…akt, Bcl-2.

- Tumor cell or Tumor-associated-macrophage production of local factors (TGF-β, IL-10) that suppress T cell responses and DCs (VEGF, and TGF, IL-10)

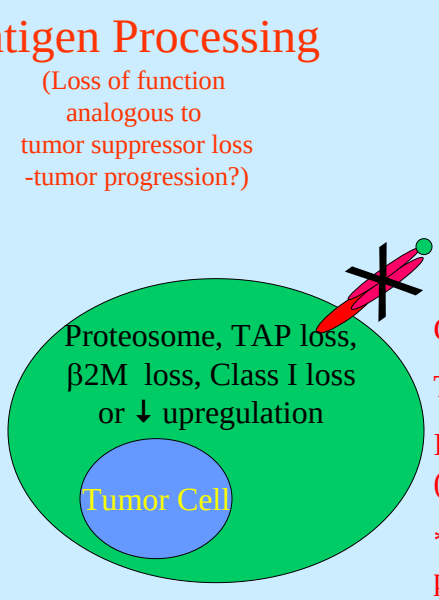
# More Bad News/Tumor Evasion Resistance to Effector Response

- 2 pages of problems…not good
- FasL expression on tumor cells may induce cell death of Fas + T cells.
- Conventional T cells may be suppressed by Treg cells preferentially induced or recruited by tumor.
- (early clinical promise with Treg depleting approaches and anti-CTLA4 antibodies).
- Antigen modulation (antibody-mediated endocytosis of surface antigen)
- Loss of tumor antigen expression: Tumor heterogeneity (need to target multiple antigens)-and possibly proteins essential for transformation/growth.
- Loss of antigen presentation capacity by tumor



## Alterations in Antigen Processing

(Loss of function analogous to tumor suppressor loss -tumor progression?)




Frequency

Class I loss/↓ reg'n 31-70%

TAP/Proteosome(LMP2,7)10-80%

IFN-gammaR signaling defect (rare)

*associated with metastatic and poor prognostic lesions

## Immunological Intervention: Early Successes

- Cooley's toxin (gram + bacteria injected into tumor sites): local inflammatory rxn and systemic toxicity (fever, sepsis syndrome) associated with occasional tumor remissions (bacterial product induced production of IL-12, IFN-γ, TNFα – enhanced antigen presentation??)
- Systemic cytokines (IL-2, IL-12, IFN-α) 1980-90's. Occasional responses (shrinkage in 5-15% of cases) with high toxicities. Higher responses for IFN-α in CML and hairy cell leukemia; CML remissions associated with anti-PR1 (proteinase in CML cells) T cell responses.

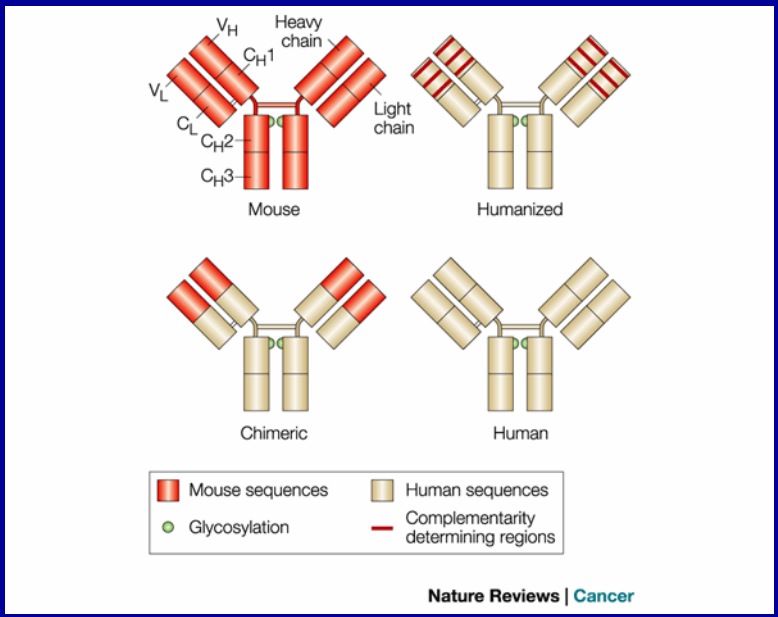
## Strategies for induction of anti-tumor Immune Responses

-Passive-

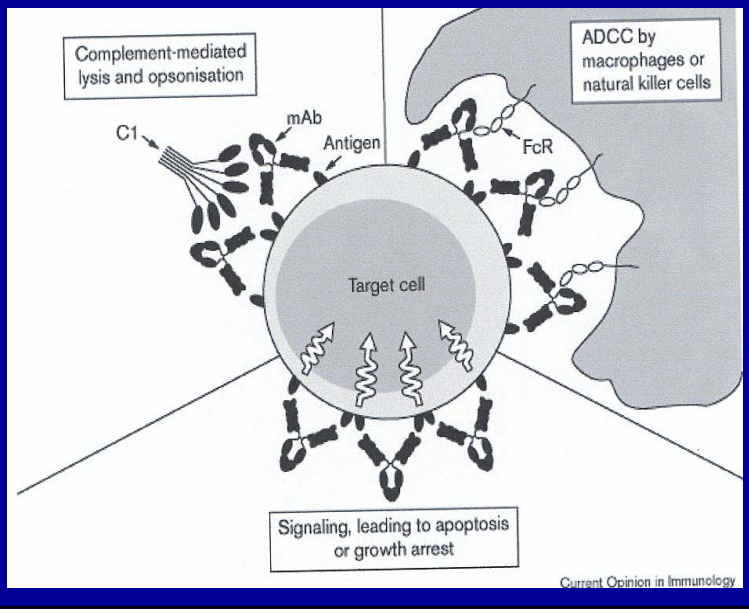
- **Adoptive transfer of T cells:** Antigenic specific T cell clones-requires HLA-restricted "customized" therapy or cytokine-enhanced antigen-non-specific T cells (LAK cells). Has worked for EBV lymphoproliferative disorders.

- **Monoclonal and engineered antibodies:**

1. Humanized/chimeric mAbs: Herceptin (anti-HER2), Rituxan (anti-CD20), anti-idiotype (custom therapy), anti-EGFR (Erbitux), CAMPATH (anti-CD52), anti-VEGF (targets neovasculature, Avastin).
2. Immune conjugates ("smart bombs") mAb-toxin (Mylotarg: anti-CD33 calicheamicin), mAb-chemo, mAb-isotope (anti-CD20 Zevalin and Bexxar).



## Potential Cytotoxic Mechanisms of Anti-Tumor Antibodies

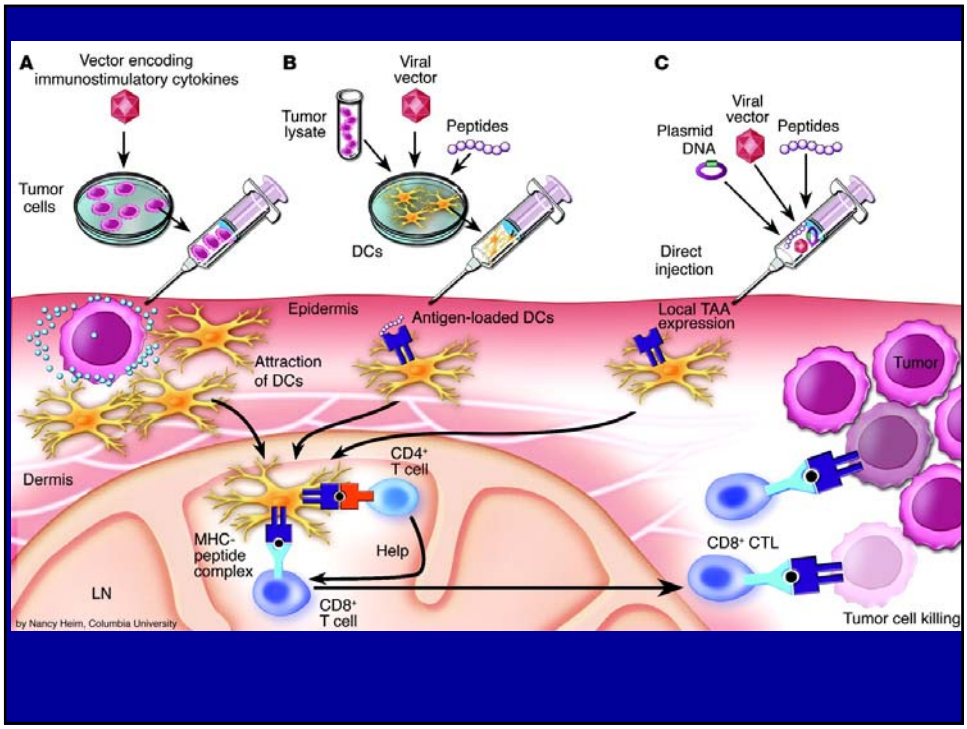



## Strategies for induction of anti-tumor Immune Responses

ACTIVE IMMUNIZATION

Goal is to define tumor antigens and then use them in an immunostimulatory fashion.

How to induce immune response and break tolerance: Essentially "the dirty little secret" of immunologists- ***the adjuvant effect***;effective immunization usually requires mixing antigen with agents which promote uptake of antigen by APCs as well as activate and recruit APCs to vaccine site (e.g. Alum or Complete Freund's Adjuvant: mineral oil/water emulsion + heat killed bacillus).




# Tumor Immunology: Summary

1) Immunological recognition of tumor occurs.

2) Tumors emerge in individuals having overcome immunological surveillance.

3) Evasion mechanisms include reduced tumor antigen presentation and local immunoregulatory factors: inhibitory cytokines and cells.

4) Reversal of tolerogenic response is goal of immunotherapy
Passive immunization (antitumor antibodies, adoptive T cell therapy).
Active immunization (vaccine=antigen plus adjuvant).
The goal is to induce antigen specific effector T cells while eliminating regulatory negative immunoregulatory pathways.