

Tumor Immunity (Clynes)

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?

More “good” news

Evidence for Immunological Surveillance in Man Cancer Incidence Increases in Immunosuppressed

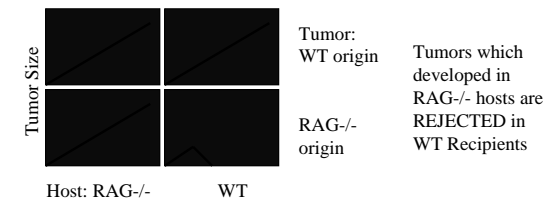
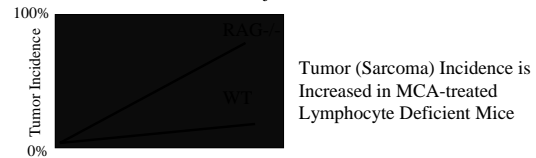
- Increased incidence of malignancies in HIV patients: EBV lymphoma, KS, squamous cell CA –but many of these are virally induced malignancies; this merely shows that eliminating a T cell response against **virial** antigens allows for the outgrowth of virally-transformed cells. Common variety neoplasms (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).

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, graft 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.

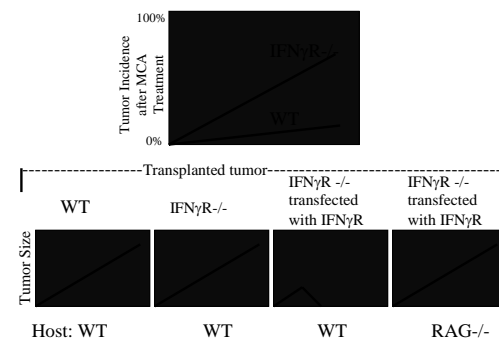
Immunosurveillance: Tumors which Evolve in Lymphocyte Deficient Hosts are Rejected in WT Mice



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.

Immune Surveillance: Tumor Cell Expression of IFN γ Receptor is Required for Lymphocyte-Mediated Tumor Rejection



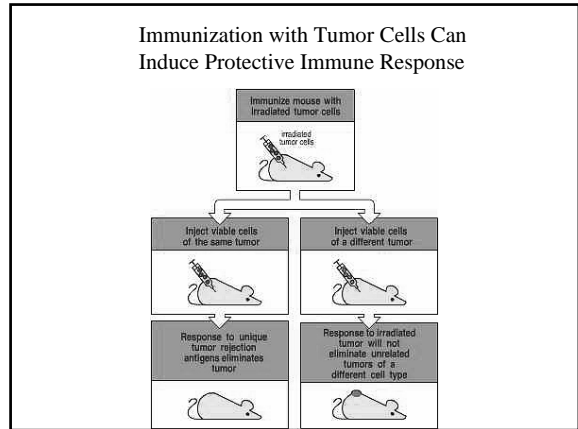
Tumor Immunity (Clynes)

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 ¹⁰ Spontaneous intracranial neoplasia ¹⁰
RAG-2 ^{-/-} × STAT1 ^{-/-} (RAG)	T, B and NKT cells	MCA-induced sarcomas ¹⁰ Spontaneous intracranial and mammary neoplasia ¹⁰
BALB/c, SCID	T, B and NKT cells	MCA-induced sarcomas ¹⁰ Spontaneous disseminated lymphomas ¹⁰
Perforin ^{-/-}	Lack of perforin	MCA-induced sarcomas ¹⁰ Spontaneous disseminated lymphomas ¹⁰
TCR β ^{0/0}	Subset of NKT cells	MCA-induced sarcomas ¹⁰ Spontaneous disseminated lymphomas ¹⁰
Anti-anti-GPI antibody	NK cells and activated macrophages	MCA-induced sarcomas ¹⁰
Anti-NK1.1 antibody	NK and NKT cells	MCA-induced sarcomas ¹⁰
Anti-Thy1 antibody	T cells	MCA-induced sarcomas ¹⁰
αβ T cell ^{-/-}	αβ T cells	MCA-induced sarcomas ¹⁰
γδ T cell ^{-/-}	γδ T cells	MCA-induced sarcomas ¹⁰
STAT1 ^{-/-}	Insensitive to IFN-γ and IFN-α/β	MCA-induced sarcomas ¹⁰ Wider tumor spectrum in STAT1 ^{-/-} × p53 ^{-/-} (ref. 41)
IFNGR1 receptor ^{-/-}	Insensitive to IFN-γ	MCA-induced sarcomas ¹⁰ Wider tumor spectrum in IFN-γ receptor × p53 ^{-/-} (ref. 41)
IFN-γ ^{-/-}	Lack of IFN-γ	MCA-induced sarcomas ¹⁰ CSF1R ^{-/-} Spontaneous disseminated lymphomas ¹⁰ BALB/c Spontaneous lung adenocarcinomas ¹⁰
Perforin ^{-/-} × IFN-γ ^{-/-}	Lack of perforin and IFN-γ	MCA-induced sarcomas ¹⁰ Spontaneous disseminated lymphomas ¹⁰
IL-12 ^{-/-} WT + IL-12	Lack of IL-12 Exogenous IL-12	MCA-induced sarcomas ¹⁰ Lower incidence of MCA-induced sarcomas ¹⁰

Multiphasic/chronic-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.

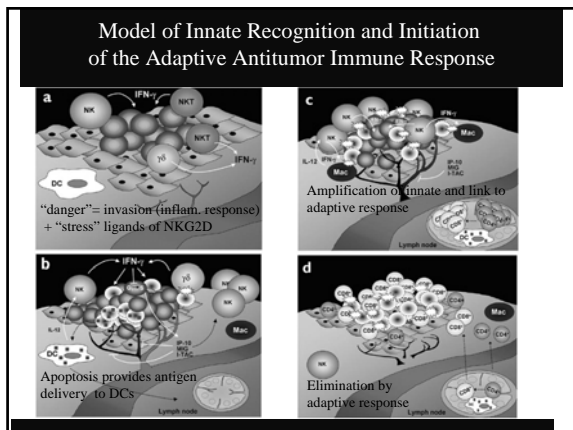
Evidence that IFN-induced antigen presentation by tumor cells provides immunity (as with viral immunity). IFN-γR^{-/-} tumors grow in WT mice, unless transfected with TAP. Highly immunogenic tumors emerge in RAG^{-/-} mice; these tumors grow in RAG^{-/-} (in absence of immune selective pressure) but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

Tumor Antigens Are Unique to Individual Tumors

Immunized Tumor

	A	B	C	D	E	F	G	H	I
A									
B									
C									
D									
E									
F									
G									
H									
I									

Legend: Protection, No protection



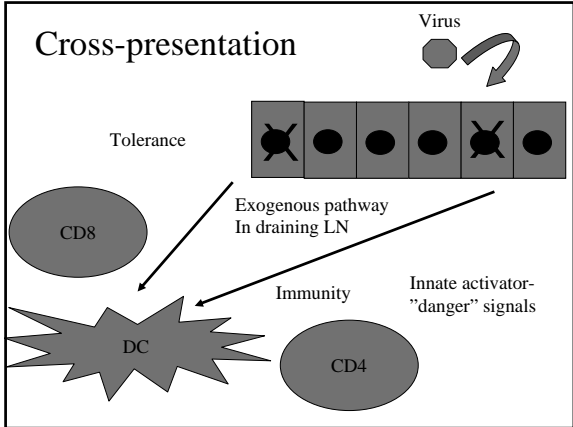
Candidate Tumor Antigens

Antigen Class	Antigen	Advantages/ Disadvantages
Whole Cell	Protein lysate or tumor RNA based expression	Universal (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. Antigenic modulation or loss (overcome by attacking multiple targets and antigens required for transformed phenotype).

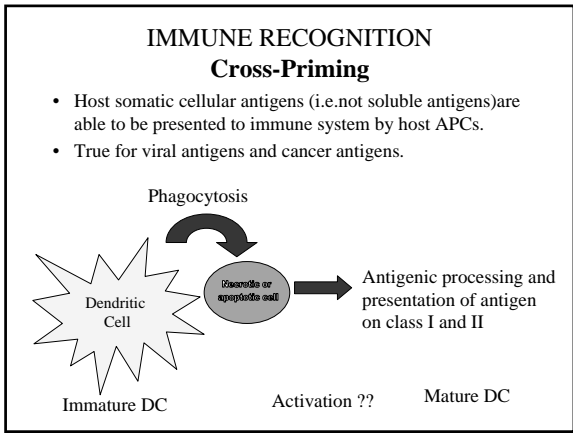
Tumor Immunity (Clynes)

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



IMMUNE RECOGNITION

Tumor Evasion

Tumor cells are poorly immunogenic

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

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 M ϕ , 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

NK Cells Can Recognize Class I Negative Cells and Induce Tumor Lysis and Apoptosis

Granule exocytosis: Perforin/granzyme

Class I

Fas - FasL

Yet, class I loss is common in cancer. Lack of activation of NK receptors? Cytokine “milieu”?

IMMUNE RECOGNITION

Cross-Priming: Induction of Anti-tumor T cell response

CD28 CTL

CD8 TCR

IL-2

CD4 TCR CD4 Th1

CD40L

Class I + peptide

Class II + peptide

CD40

Ag Processing/presentation of peptides

Endocytosis/phagocytosis

Tumor Cell

Provide T_{H1} or 2 Help for B cell

Ab Responses

Effector Mechanisms

Macrophages are Cell-Mediated Effectors

TNF (+ other TNF-family members) NO, O₂*, proteases

CD4 TCR CD4 Th1

CD40L

Class II + peptide

CD40

Cytokine-Mediated Activation

IFN- γ

GM-CSF

TNF

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

Antibody Bound Targets Induce Myeloid Cell Tumor Cytotoxicity Through Fc Receptors +/- Complement Receptors

ADCC, phagocytosis, release of inflammatory mediators (NO, O₂*, proteases, TNF, etc.,)

Tumor Cell

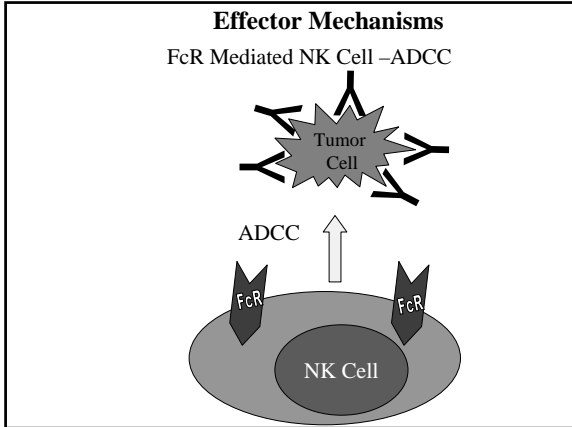
CR1

FcR

Macrophage

C3b

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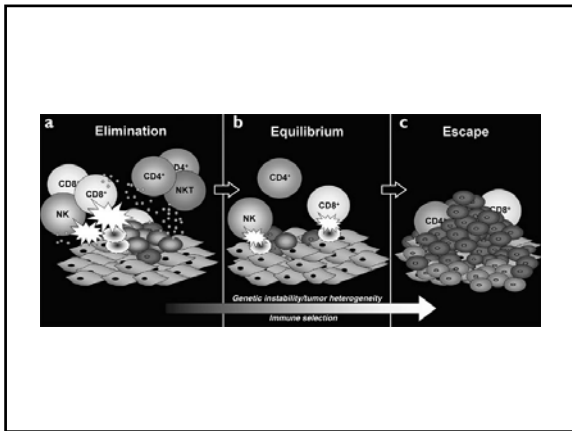
Bad News/Tumor Evasion Resistance to Effector Response

- 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)



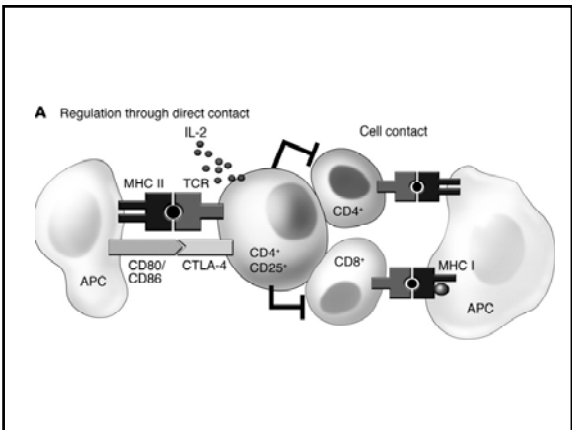
More Bad News/Tumor Evasion Resistance to Effector Response

- 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

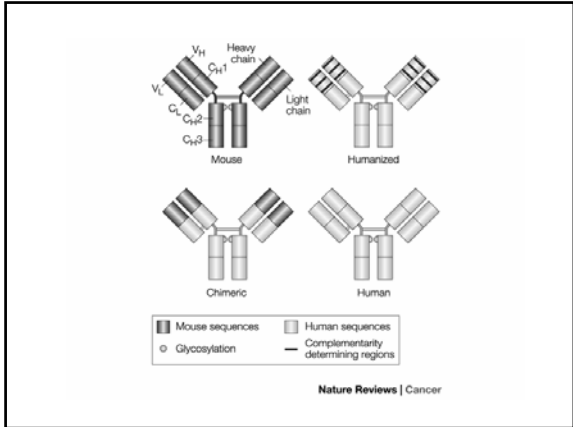
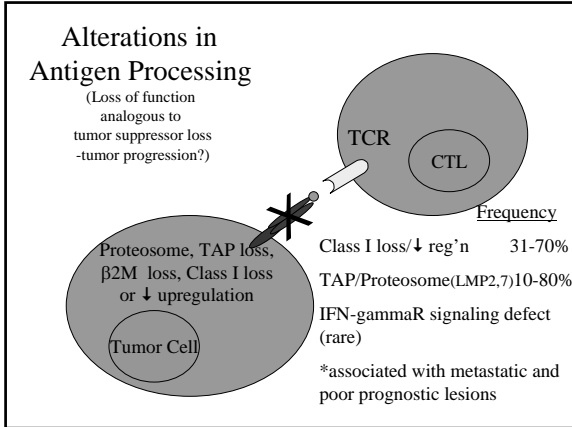
Tumor Evasion: Two separate problems

- 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).

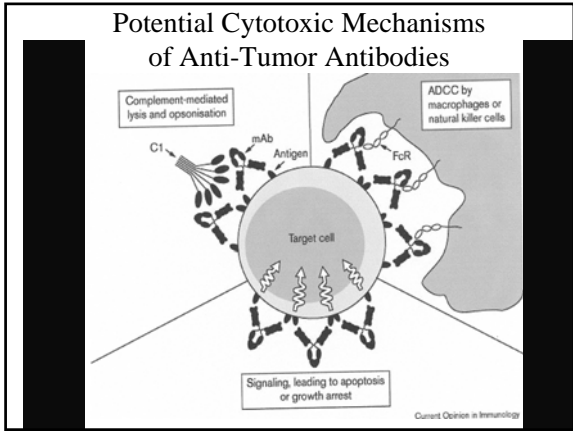


Tumor Immunity (Clynes)



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 , TNF α – 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-idiotypic (custom therapy), anti-EGFR (Erbix), 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).

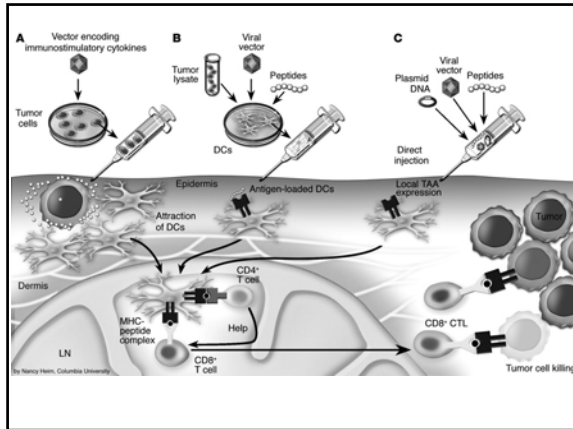
Strategies for induction of anti-tumor Immune Responses

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 Immunity (Clynes)



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.