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, 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.

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.

- 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.

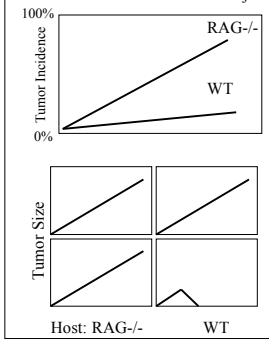
More "good" news

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.,) may be seen with increased frequency as HIV patients live with their disease longer
- 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



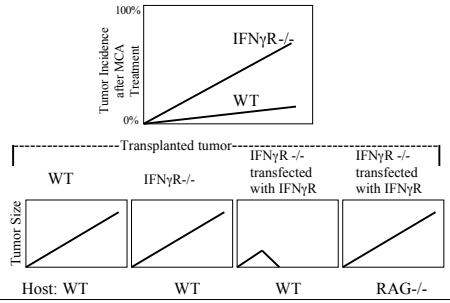
Tumor (Sarcoma) Incidence is Increased in MCA-treated Lymphocyte Deficient Mice

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[48] Spontaneous intestinal neoplasia[49]
RAG-2$^{-/-}$ × STAT1$^{-/-}$ (Rag3ko)	T, B and NKT cells Insensitive to IFN-γ and IFN-α/β	MCA-induced sarcomas[48] Spontaneous intestinal and mammary neoplasia[49]
BALB/c SCID	T, B and NKT cells	MCA-induced sarcomas[100]
Perforin$^{-/-}$	Lack of perforin	MCA-induced sarcomas[47,48,66] Spontaneous disseminated lymphomas[47,49]
TCR Jα281$^{-/-}$	Subset of NKT cells	MCA-induced sarcomas[47,48,100]
Anti-asialo-GM1 antibody	NK cells and activated macrophages	MCA-induced sarcomas[101]
Anti-NK1.1 antibody	NK and NKT cells	MCA-induced sarcomas[46,102]
Anti-Thy1 antibody	T cells	MCA-induced sarcomas[46,100]
αβ T cell$^{-/-}$	αβ T cells	MCA-induced sarcomas[66]
γδ T cell$^{-/-}$	γδ T cells	MCA-induced sarcomas[66] DMBA/TPA-induced skin tumors[103]
STAT1$^{-/-}$	Insensitive to IFN-γ and IFN-α/β	MCA-induced sarcomas[47,48] Wider tumor spectrum in STAT1$^{-/-}$ × p53$^{+/-}$ (ref. 41)
IFNGR1 receptor$^{-/-}$	Insensitive to IFN-γ	MCA-induced sarcomas[47,48] Wider tumor spectrum in IFN-γ receptor$^{-/-}$ × p53$^{+/-}$ (ref. 41)
IFN-γ$^{-/-}$	Lack of IFN-γ	MCA-induced sarcomas[47] C57BL/6: Spontaneous disseminated lymphomas[47] BALB/c: Spontaneous lung adenocarcinoma[49]
Perforin$^{-/-}$ × IFN-γ$^{-/-}$	Lack of perforin and IFN-γ	MCA-induced sarcomas[47] Spontaneous disseminated lymphomas[47]
IL-12$^{-/-}$	Lack of IL-12	MCA-induced sarcomas[104]
WT + IL-12	Exogenous IL-12	Lower incidence of MCA-induced sarcomas[104]

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

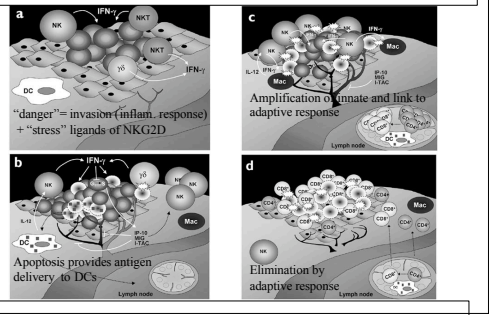
More good news/ Evidence for Immunological Surveillance

- 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.

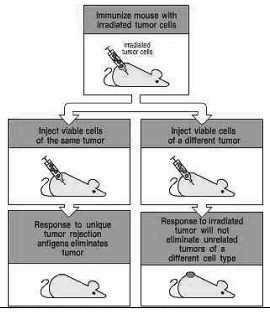
Evidence that IFN-induced antigen presentation by tumor cells provides immunity (as with viral immunity). IFN-γR -/- tumors grow in WT mice, unless transfected with TAP. Highly immunogenic tumors emerge in RAG -/- mice; these tumors grow in RAG -/- (in absence of immune selective pressure) but are rejected 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. (which in turn activates STAT1 in the tumor and in immune cells)

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

Immunized Tumor

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

☐ 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).

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.,

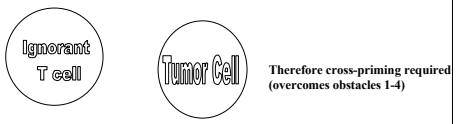
- **Unique Tumor Antigens** (requires tumor specific therapy) Antigenic modulation would potentially interfere with malignant phenotype.

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 immunogeneic

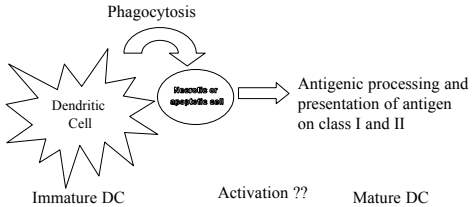


Therefore cross-priming required (overcomes obstacles 1-4)

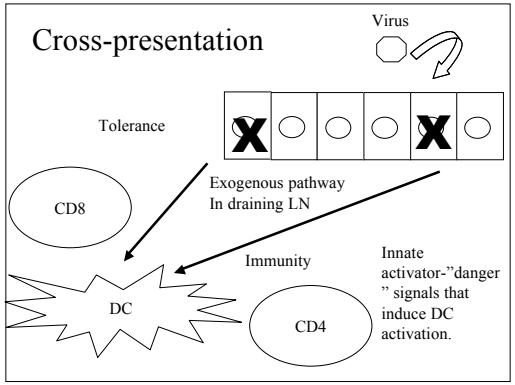
Poor APCs
1) Often no class I
2) No class II
3) No costimulatory molecules
4) Few adhesion molecules
5) Antigenically largely self

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.

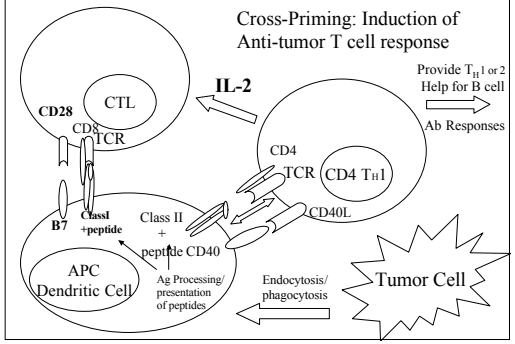


Cross-presentation



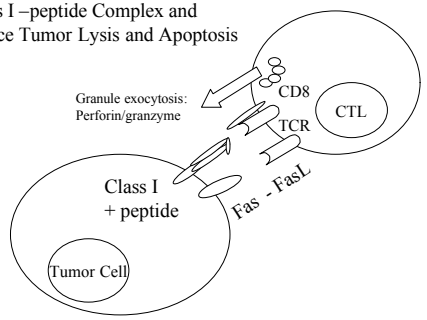
IMMUNE RECOGNITION

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



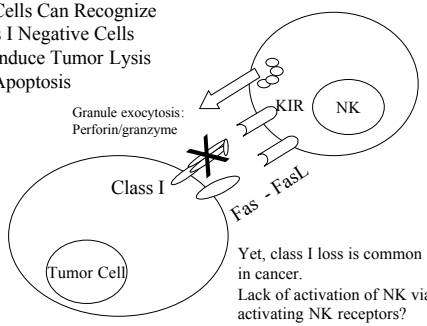
Effector Mechanisms

CD8 CTL Can Recognize
Class I –peptide Complex and
Induce Tumor Lysis and Apoptosis



Effector Mechanisms

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

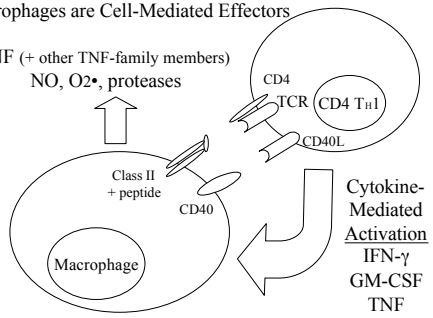


Yet, class I loss is common in cancer.
Lack of activation of NK via activating NK receptors?
Cytokine "milieu"?

Effector Mechanisms

Macrophages are Cell-Mediated Effectors

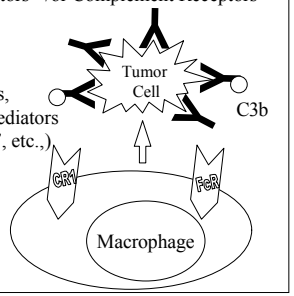
TNF (+ other TNF-family members)
NO, O2•, proteases



Cytokine-Mediated Activation
IFN-γ
GM-CSF
TNF

Effector Mechanisms

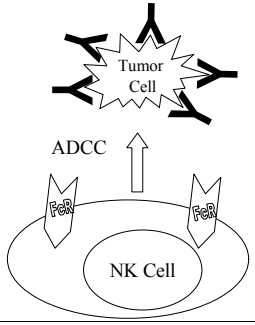
Antibody Bound Targets Induce Myeloid Cell Tumor Cytotoxicity Through Fc Receptors +/or Complement Receptors



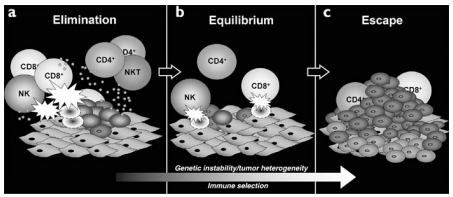
ADCC, phagocytosis,
release of inflammatory mediators
(NO, O2•, proteases, TNF, etc.,)

Effector Mechanisms

FcR Mediated NK Cell –ADCC



Tumor Evasion of the Immune Response



Tumor Evasion: Two separate problems

- Tumor antigens are not recognized by immune response-poorly immunogenic *(*Immunologically *ignorant)*.
- Tumors and tumor environment are resistant to or inhibit immune cytotoxic responses. (active *suppression*— either dampen "priming" or inhibit/resist effector cell function).

Bad News/Tumor Evasion Resistance to Effector Response

Tumor Cell Properties

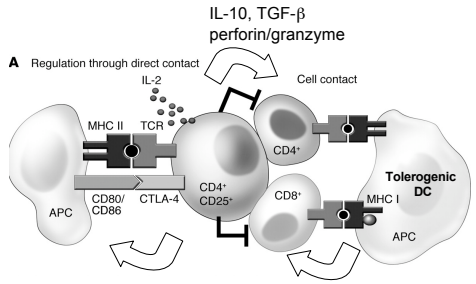
- Intrinsic resistance
  Upregulation of genetic "survival" pathways (**anti-apoptotic genes**), e.g. akt, Bcl-2. etc.,
  Resistance to death receptor pathways: Reduction of Fas receptor or enhanced expression of c-FLIP by tumors may render tumors resistance to fas-mediated apoptosis. Similarly, tumors commonly lose TRAIL receptors or express "decoy" receptors.
  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

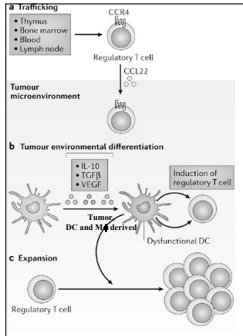
More Bad News/Tumor Evasion Resistance to Effector Response

2 pages of problems…not good

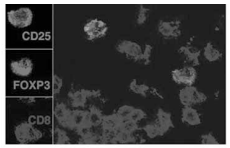
Local Inhibitory Factors in the Tumor Stroma produced by 1) tumor cells themselves or by 2) stromal T cells or macrophages

- Tumor cell or Tumor-associated-macrophage production of local factors (TGF-β, IL-10) that suppress T cell responses and DCs (VEGF, and TGF-β, IL-10)
- Conventional T cells and DCs may be suppressed by **Treg cells** preferentially induced or recruited by tumor. (early clinical promise with Treg depleting approaches and anti-CTLA4 antibodies).



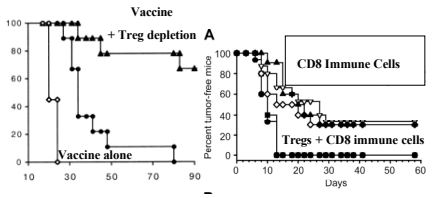


FOXP3+ Tregs in human tumor (ovarian tumor ascites)



T regulatory cells are also present in draining lymph node. Thereby inhibiting both T cell priming (in the lymph node) and effector T cell responses (in the tumor tissue)

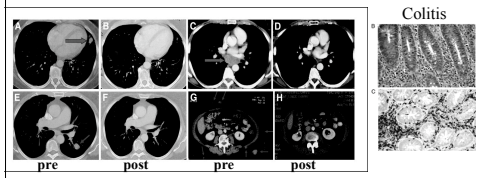
Regulatory T cells Suppress Tumor Immunity



Autoimmunity Correlates With Tumor Regression in Patients With Metastatic Melanoma Treated With Anti-CTLA-4

Grade 3/4 autoimmunity 30%
(colitis, uveitis, hypopophysitis, thyroiditis, hepatitis, dermatitis)

Tumor response rate 15%

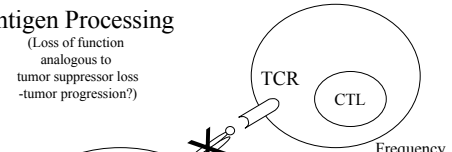


Colitis

Attia, P. et al. J Clin Oncol; 23:6043-6053 2005

Alterations in Antigen Processing

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



Frequency

Proteosome, TAP loss, β2M loss, Class I loss or ↓ upregulation — Class I loss/↓ reg'n 31-70%

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

Tumor Cell

IFN-gammaR signaling defect (rare)

*associated with metastatic and poor prognostic lesions

Strategies for induction of anti-tumor Immune Responses

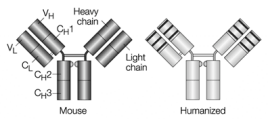
-Passive-

- **Adoptive transfer of autologous tumor Ag-specific T cells:** "customized" therapy: Requires ex vivo expansion of cytokine-enhanced antigen-specific memory T cells.

  Has worked for EBV lymphoproliferative disorders and melanoma.

- **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).

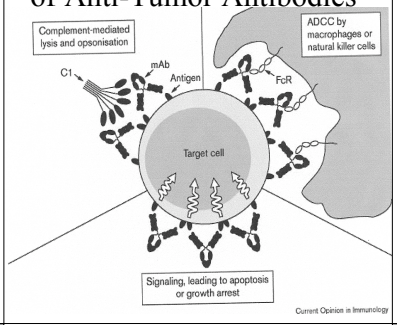


Nature Reviews | Cancer

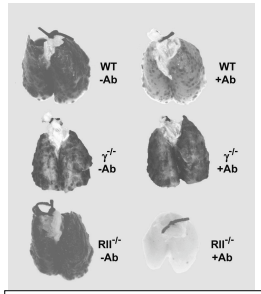
Therapeutic Antibodies in Oncology

mAb	Construct	Isotype	Target
Rituxan™ ***(rituxamab)***	**Chimeric**	**IgG1**	**CD20**
Herceptin™ ***(trastuzumab)***	**Humanized**	**IgG1**	**HER-2**
Campath™ ***(alemtuzumab)***	**Humanized**	**IgG1**	**CD52**
Erbitux™ ***(cetuximab)***	**Chimeric**	**IgG1**	**EGFR**
Avastin™ ***(bevacisamab)***	**Humanized**	**IgG1**	**VEGF- A**
Bexxar (Tositumomab) 1131	**Mouse**	**IgG1**	**CD20**
Zevalin™ ***(Ibritumomab tiuxetan) Ytrium90***	**Mouse**	**IgG1**	**CD20**
Mylotarg™ ***(aemtuzumab ozogamicin)***	**Humanized**	**IgG4**	**CD33**
ABX-EGF	**Human**	**IgG2**	**EGFR**

Potential Cytotoxic Mechanisms of Anti-Tumor Antibodies



Fc Receptors Modulate Anti-Melanoma TA99 Monoclonal Antibody Efficacy



Normal Mouse

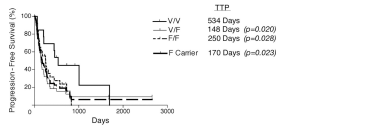
Mouse without Activating Fc receptors

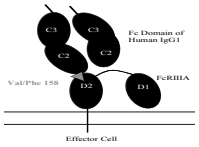
Mouse without Inhibitory Fc receptors

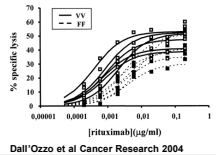
Clynes, Takechi, Morai, Houghton, Ravetch, PNAS, 1998

Rituxan Clinical Responses in Follicular Lymphoma are Associated with High Affinity FcR Polymorphic Alleles

Weng and Levy, JCO, Vol. 21 (2003)







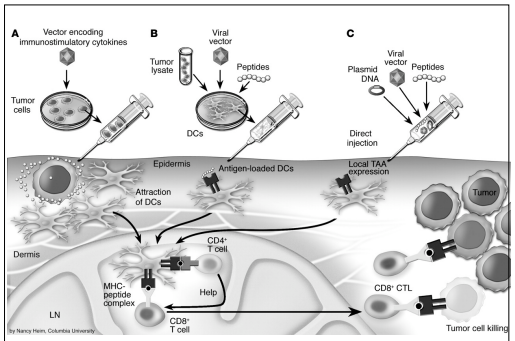
Dall'Ozzo et al Cancer Research 2004

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).



How to present antigen: clinical trials

- Systemic cytokines (e.g.IFNα); upregulate HLA/antigen processing, mature and activate APC
- Whole cell and adjuvant
- Tumor antigen protein or peptide and adjuvant
- Peptide and cytokines
- Turn cancer cell into an APC or a recruiter of APCs: transfect/infect tumor with costim. gene (B7) or with cytokine gene (GM-CSF), DC tumor cell fusion.
- Gene gun (DNA vaccination:tumor specific gene+/-costimulatory+/-cytokine genes)
- Autologous DC's "pulsed" with protein, peptides etc. Attempts to deliver tumor peptide for cytosolic class I loading in activated DCs.

Tumor Immunology: Summary

1) Immunological recognition of tumor occurs. Tumor specific T cells are found in patients, but are ineffective.
Classify the types of tumor antigens thus far identified.

2) Tumors have emerged in individuals having successfully overcome immunological surveillance.
Experimental data from mice are provided showing that immunological surveillance prevents tumor development and there are examples in humans in which cancers emerge in immunodeficient individuals.

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).
<u>The goal is to induce antigen specific effector T cells while eliminating regulatory negative immunoregulatory pathways.</u>