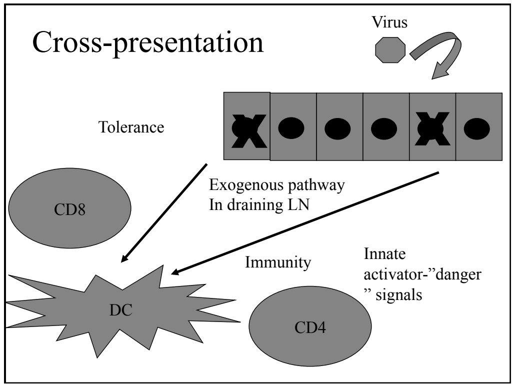
Cross-presentation



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. 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.

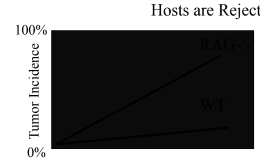
More "good" news

Evidence for Immunological Surveillance in Man

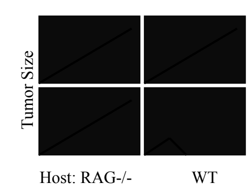
Cancer Incidence Increases in Immunosuppressed

- 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.,) 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

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

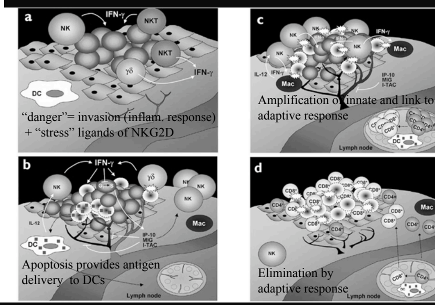
Phenotype or depletion	Immunodeficiency	Tumor susceptibility
RAG-2$^{-/-}$	T, B and NKT cells	MCA-induced sarcomas[49] Spontaneous intestinal neoplasia[49]
RAG-2$^{-/-}$ × STAT1$^{-/-}$ (RkSk)	T, B and NKT cells Insensitive to IFN-γ and IFN-α/β	MCA-induced sarcomas[49] Spontaneous intestinal and mammary neoplasia[49]
BALB/c SCID	T, B and NKT cells	MCA-induced sarcomas[100]
Perforin$^{-/-}$	Lack of perforin	MCA-induced sarcomas[42,45,46] Spontaneous disseminated lymphomas[43,47]
TCR Jα281$^{-/-}$	Subset of NKT cells	MCA-induced sarcomas[42,46,100]
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] DMBA/TPA-induced skin tumors[50]
STAT1$^{-/-}$	Insensitive to IFN-γ and IFN-α/β	MCA-induced sarcomas[41,49] Wider tumor spectrum in STAT1$^{-/-}$ × p53$^{-/-}$ (ref. 41)
IFNGR1 receptor$^{-/-}$	Insensitive to IFN-γ	MCA-induced sarcomas[41,49] Wider tumor spectrum in IFN-γ receptor$^{-/-}$ × p53$^{-/-}$ (ref. 41)
IFN-γ$^{-/-}$	Lack of IFN-γ	MCA-induced sarcomas[42] C57BL/6: Spontaneous disseminated lymphomas[43] BALB/c: Spontaneous lung adenocarcinoma[43]
Perforin$^{-/-}$ × IFN-γ$^{-/-}$	Lack of perforin and IFN-γ	MCA-induced sarcomas[42] Spontaneous disseminated lymphomas[43]
IL-12$^{-/-}$	Lack of IL-12	MCA-induced sarcomas[46]
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.

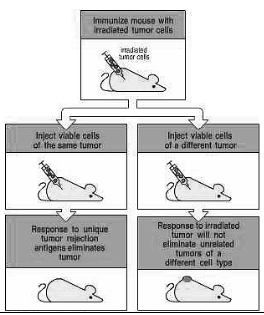
More good news/ Evidence for Immunological Surveillance

- 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.
- Highly immunogenic tumors emerge in RAG -/- mice spontaneously; these tumors grow in RAG -/- (in absence of immune selective pressure) but are rejected in WT mice (in presence of normal immune response).

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

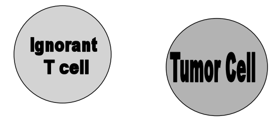
Candidate Tumor Antigens..many more to come through genomics

- **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

IMMUNE RECOGNITION

Tumor cells are poorly immunogeneic



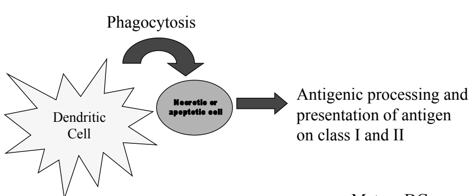
Therefore cross-priming required (overcomes obstacles 1-4)

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

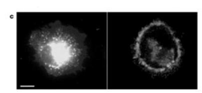
IMMUNE RECOGNITION

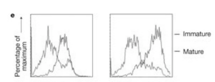
Cross-Priming

- 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.



DC Maturation





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, StAg,
- Inflammatory Cytokines: TNF, IFN, (products of either Mϕ, NK or T cells)

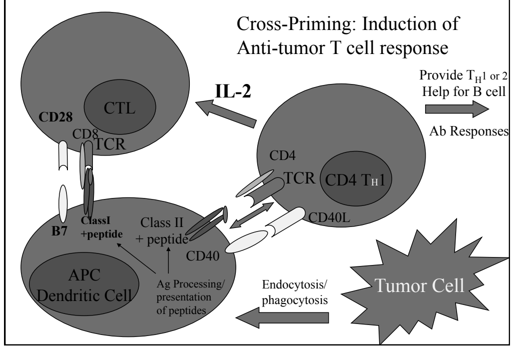
Effective antigen presentation by "cross-priming" enhanced by DC activation/maturation (CD40L, TNF, others)

- Peripheral immature DCs migrate to LN upon activation by antigen/cytokines where they may encounter T cells.

Maturation marked by

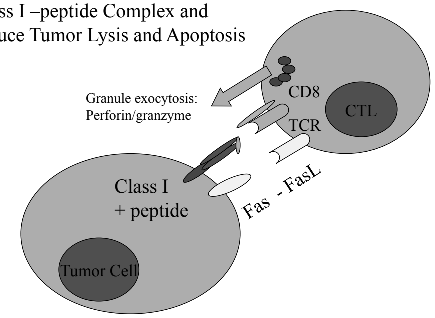
- Upregulation of antigen processing and surface expression of class I and II molecules (signal 1)
- Upregulation of co-stimulatory molecules CD40, B7 (CD80,86), adhesion molecules (ICAM-1) and cytokines for interaction and activation of antigen-specific T cells (signal 2).

IMMUNE RECOGNITION



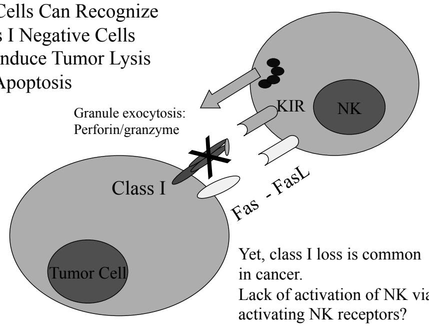
Effector Mechanisms

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



Effector Mechanisms

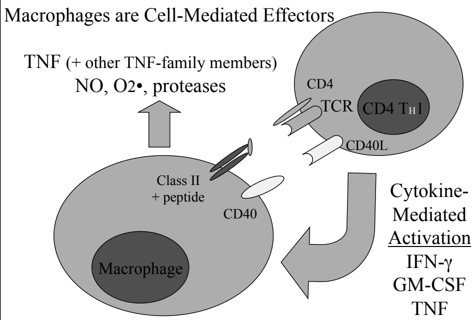
NK Cells Can Recognize
Class I Negative Cells
and Induce Tumor Lysis
and Apoptosis



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

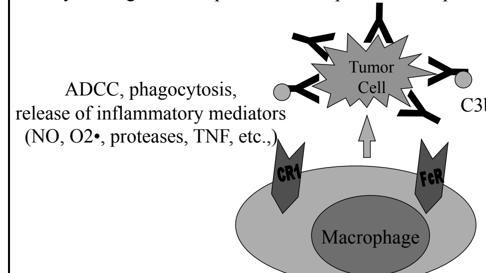
Effector Mechanisms

Macrophages are Cell-Mediated Effectors



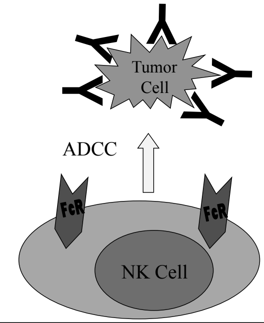
Effector Mechanisms

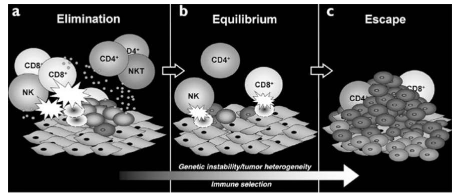
Antibody Bound Targets Induce Myeloid Cell Tumor Cyto-toxicity Through Fc Receptors +/or Complement Receptors



Effector Mechanisms

FcR Mediated NK Cell –ADCC





Tumor Evasion: Two separate problems

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

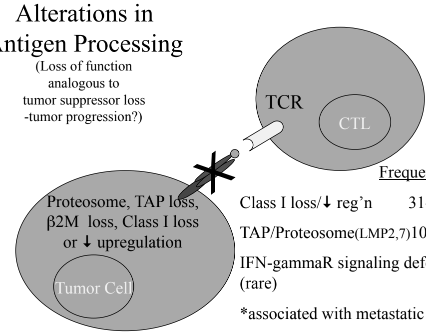
*(*Immunologically *ignorant)*.

- Tumors are resistant to or inhibit immune cytotoxic responses.

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

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

Bad News/Tumor Evasion

Resistance to Effector Response

(Tumor Cell)

- **<u>Loss of antigen presentation capacity by tumor</u>**
- 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.

  <u>*Upregulation of "survival" pathways…akt, Bcl-2.</u>
- Antigen modulation (antibody-mediated endocytosis of surface antigen)
- **Loss of tumor antigen expression: <u>Tumor heterogeneity</u> (need to target multiple antigens)-and possibly proteins essential for transformation/growth.**

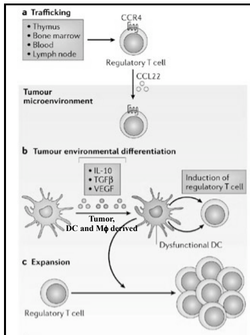
More Bad News/Tumor Evasion

Resistance to Effector Response

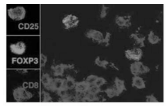
(Tumor Stroma)

- 2 pages of problems…not good
- Tumor cell or Tumor-associated-macrophage production of local factors that suppress T cell responses (TGF-β, IL-10) and DCs (VEGF, and TGF, IL-10).
- Conventional T cells may be suppressed by Treg cells preferentially induced or recruited by tumor.

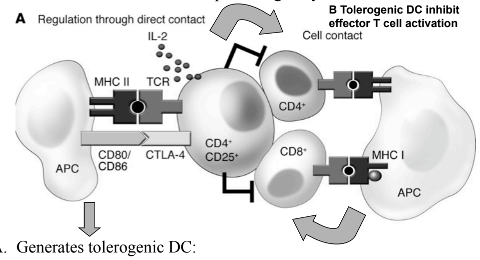
**(early clinical promise with Treg depleting approaches and/or anti-CTLA4 antibodies).



FOXP3+ Tregs in hunam tumor (ascites), also in draining lymph node.



IL-10, TGF-β
perforin/granzyme



A. Generates tolerogenic DC:

a) "immature"

b) Producers of tolerogenic cytokines (IL-10, TGF-beta) & IDO (effects tryptophan metabolism)

IL-10, tryptophan metabolites

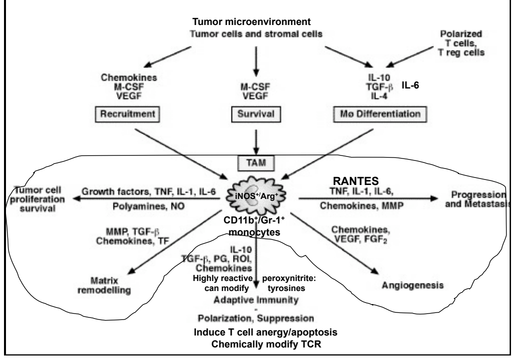
Cancer and Inflammation: Seed and Soil Hypothesis

--Stromal inflammation as tumor "promoter"

--"tolerogenic" healing/remodeling/repair

Malignancy	Inflammatory stimulus
Bladder cancer	Schistosomiasis
Gastric cancer	H. pylori-induced gastritis
MALT lymphoma	H. pylori
Hepatocellular CA	Hepatitis virus (B and C)
Kaposi's sarcoma	HHV8
Lung CA	Silica, Asbestos
Mesothelioma	Asbestos
Ovarian cancer	Salpingitis/talc/ovulation/endometriosis
Colorectal cancer	Inflammatory bowel disease
Esophageal cancer	Barrett's metaplasia
Papillary thyroid CA	Thyroiditis
Prostate cancer	Prostatitis

Tumor Associated Macrophages/ Myeloid Suppressor Cells



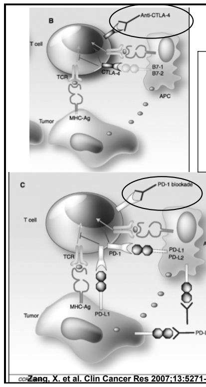
Macrophage Products that Drive Tumorogenesis

- **Growth and survival**
  Basic FGF, EGF, hepatocyte growth factor, PDGF, IL-6, TNF, polyamines, PGE2
- **Angiogenesis**
  VEGF, MMP-9, IL-1, IL-8, urokinase-type plasminogen activator (uPA), CXCL1, CXCL8, HIF-1, HIF-2, PGE2
- **Tissue invasion and metastases**
  Chemokines, PGE2, matrix metalloproteinases, uPA, plasmin
- **Mutations**
  Superoxide, peroxynitrite
- **Inhibition of T cell responses**
  IL-10, TGF-β, indoleamine-2,3-dioxygenase, PGE2, superoxide, peroxynitrite, arginase

On the near horizon: Removing immuno-inhibitory pathways

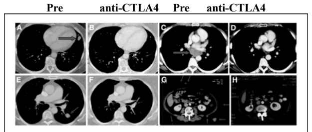
- **Anti-CTLA4 Abs:** 15% clinical response in melanoma, prostate, etc., Autoimmunity seen in many patients. Combined therapy with tumor vaccines ongoing.
- **Treg depletion** (IL-2 Diptheria toxin conjugate)
- **Anti-PD-1:** Reversal of T cell exhaustion?

Removal of Inhibitory Regulation



Zang, X. et al. Clin Cancer Res 2007;13:5271-5279

Pre	Peptide vacc.+ anti-CTLA4	Pre	Peptide vacc.+ anti-CTLA4



Activating/Inhibitory Co-Stimulation

APC/Tumor		T cell
	+	ICOS
		CD28
CD80/CD86	–	CTLA-4
PD-L1	–	
PD-L2		PD-1

Strategies for induction of anti-tumor Immune Responses

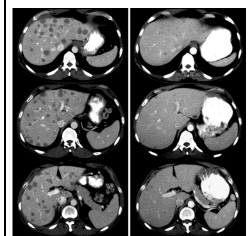
-Passive-

- **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"):

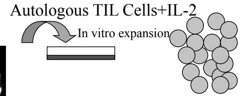
tumor-targeted antibodies can deliver toxic payloads. mAb-toxin (Mylotarg: anti-CD33 calicheamicin), mAb-chemo, mAb-isotope (anti-CD20 Zevalin and Bexxar).

Passive AdoptiveT Cell Immunotherapy in Metastatic Melanoma



Before | 1 month after T cell Transfer

Autologous TIL Cells+IL-2



Infuse 10 billion cells

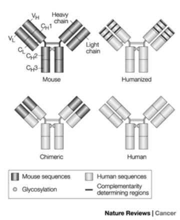
Lymphodepleted patients (by chemotherapy)

Persistent expansion of tumor-specific T cells in patients

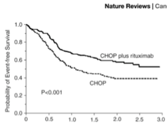
18/35 objective responses and 4 complete responses.

Rosenberg, Steven A. and Dudley, Mark E. (2004) Proc. Natl. Acad. Sci. USA 101, 14639-14645

Antibody Therapy in Cancer

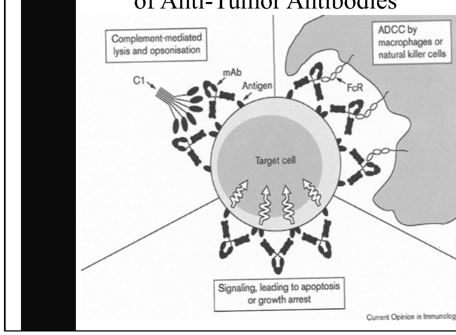


Survival with Lymphoma

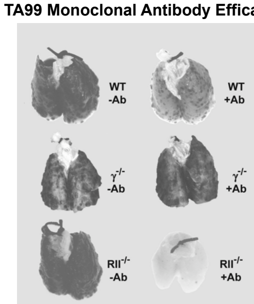


Chemo (CHOP) **+ anti-CD20 mAb** vs. Chemo (CHOP) alone

Potential Cytotoxic Mechanisms of Anti-Tumor Antibodies



Fc Receptors Modulate Anti-Melanoma TA99 Monoclonal Antibody Efficacy



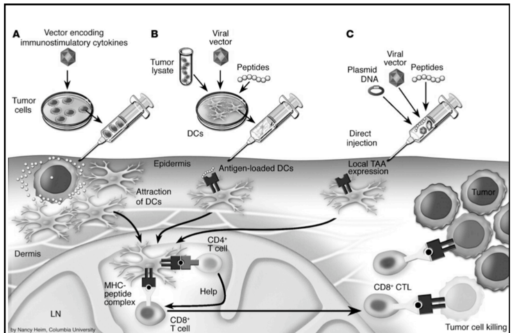
[redacted]ature Medicine 2000

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 both promote uptake of antigen by APCs as well as activate and recruit APCs to vaccine site (e.g. **classic** adjuvants: Alum or Complete Freund's Adjuvant: mineral oil/water emulsion + heat killed bacillus; **molecular** adjuvants: TLR ligands, CD40L).



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.

2) Tumors emerge in individuals having successfully overcome immunological surveillance.

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

4) Tumor development may both be promoted by chronic inflammation and be sustained by the tolerogenic tumor:stroma microenvironment.

5) Reversal of tolerogenic response is the 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 negative immunoregulatory pathways.</u>