Cross-presentation

Virus

Tolerance

Exogenous pathway
In draining LN

Immunity

Innate activator-
"danger" signals

CD8

DC

CD4
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

- **Host:** RAG-/-
  - **Tumor Incidence:**
    - **RAG-/-**
    - **WT**

- **Tumor Size**
  - **Tumor:**
    - WT origin
    - RAG-/- origin
  - **Host:** RAG-/-
    - Tumors which developed in RAG-/- hosts are REJECTED in WT Recipients
Immune surveillance:
1. Innate system
   - NK, NKT, gamma/delta T cells
   - IFN-γ, IL-12 (APC)
2. Functional conventional T cells

<table>
<thead>
<tr>
<th>Phenotype or depletion</th>
<th>Immune deficiency</th>
<th>Tumor susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG-2−/−</td>
<td>T, B and NKT cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>RAG-2−/− × STAT1−/−</td>
<td>T, B and NKT cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>(RAG)</td>
<td></td>
<td>Spontaneous intestinal neoplasia</td>
</tr>
<tr>
<td>BALB/c SCID</td>
<td>T, B and NKT cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>Perforin−/−</td>
<td>Lack of perforin</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spontaneous disseminated lymphomas</td>
</tr>
<tr>
<td>TCR 6/281−/−</td>
<td>Subset of NKT cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>Anti-asialo-GMI antibody</td>
<td>NK cells and activated macrophages</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>Anti-NK1.1 antibody</td>
<td>NK and NKT cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>Anti-CD4 antibody</td>
<td>T cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>αβ T cell−/−</td>
<td>αβ T cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>γδ T cell−/−</td>
<td>γδ T cells</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>STAT1−/−</td>
<td>Insensitive to IFN-γ and IFN-α/β</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>IFN-γR1 receptor−/−</td>
<td>Insensitive to IFN-γ</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>IFN-γ−/−</td>
<td>Lack of IFN-γ</td>
<td>Wider tumor spectrum in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>STAT1−/− × p53− (ref. 41)</td>
</tr>
<tr>
<td>Perforin−/− × IFN-γ−/−</td>
<td>Lack of perforin and IFN-γ</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>IL-12−/−</td>
<td>Lack of IL-12</td>
<td>MCA-induced sarcomas</td>
</tr>
<tr>
<td>WT × IL-12</td>
<td>Exogenous IL-12</td>
<td>Lower incidence of MCA-induced sarcomas</td>
</tr>
</tbody>
</table>

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

“danger” = invasion (inflamm. response) + “stress” ligands of NKG2D

Apoptosis provides antigen delivery to DCs

Amplification of innate and link to adaptive response

Elimination by adaptive response
Immunization with Tumor Cells Can Induce Protective Immune Response
Tumor Antigens Are Unique to Individual Tumors

Immunized Tumor

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tumor Challenge

Protection

No protection

Tumor Immunity (Clynes)
Candidate Tumor Antigens

<table>
<thead>
<tr>
<th>Antigen Class</th>
<th>Antigen</th>
<th>Advantages/ Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Cell</td>
<td>Protein lysate or tumor RNA based expression</td>
<td>Universal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Autoimmunity may be a problem)</td>
</tr>
<tr>
<td>Antigen-Specific</td>
<td>Peptide, DNA or recombinant protein</td>
<td>“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).</td>
</tr>
</tbody>
</table>
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
<table>
<thead>
<tr>
<th>Antigen Class</th>
<th>Antigen</th>
<th>Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Specific Antigen</td>
<td>Immunoglobulin Idiotype TCR Mutant ras Mutant p53 p21-/bcr-abl fusion</td>
<td>B lymphoma, MM T cell lymphoma Colorectal, lung, bladder, Head and neck cancer Pancreatic, Colon, Lung CML, ALL</td>
</tr>
<tr>
<td>Developmental Antigens (cancer/testes genes)</td>
<td>MAGE-1, MAGE-3, GAGE family, 20 genes on the X chromosome Telomerase</td>
<td>Melanoma but also in colorectal, lung, gastric Various</td>
</tr>
<tr>
<td>Viral Antigens</td>
<td>Human Papilloma Virus EBV</td>
<td>Cervical, penile cancer Burkitt’s lymphoma, nasopharyngeal Ca, post-Tx lymphoproliferative</td>
</tr>
<tr>
<td>Tissue-specific self-antigens (Differentiation antigens)</td>
<td>Tyrosinase, gp100, trp-1, trp-2 Prostatic acid phosphatase, PSA Thyroglobulin α-Fetoprotein</td>
<td>Melanoma Prostate Thyroid Liver Cancer</td>
</tr>
<tr>
<td>Over-expressed self-</td>
<td>Her-2/neu</td>
<td>Breast and lung cancer</td>
</tr>
</tbody>
</table>
Poor APCs
1) Often no class I
2) No class II
3) No costimulatory molecules
4) Few adhesion molecules
5) Antigenically largely self

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

Tumor cells are poorly immunogeneic

Tumor Immunity (Clynes)
Tumor Immunity (Clynes)

IMMUNE RECOGNITION

Cross-Priming

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

Phagocytosis

Immature DC  Activation ??  Mature DC

Dendritic Cell

Necrotic or apoptotic cell

Antigenic processing and presentation of antigen on class I and II
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).
Tumor Immunity (Clynes)

IMMUNE RECOGNITION

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

- APC (Dendritic Cell)
  - Endocytosis/phagocytosis
  - Ag Processing/presentation of peptides

- Tumor Cell
  - Cross-Priming: Induction of Anti-tumor T cell response
  - Provide $T_{H1}$ or $T_{H2}$ Help for B cell Ab Responses

- CD4 $T_{H1}$
  - TCR
  - Class II + peptide
  - CD40
  - B7
  - CD28

- CD8 CTL
  - TCR
  - Class I + peptide
  - CD40L
  - IL-2

- CTL
  - Provide $T_{H1}$ or 2 Help for B cell Ab Responses

TCR
Tumor Immunity (Clynes)

Effector Mechanisms

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

Granule exocytosis: Perforin/granzyme

Class I + peptide

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

Granule exocytosis: Perforin/granzyme

Yet, class I loss is common in cancer. Lack of activation of NK via activating NK receptors? Cytokine “milieu”?
Macrophages are Cell-Mediated Effectors

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

Class II + peptide
CD40

Macrophage

Cytokine-Mediated Activation
IFN-γ
GM-CSF
TNF

CD4 T_{\text{H1}}
CD40L
TCR
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 Cell

ADCC

NK Cell
Tumor Immunity (Clynes)
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?)

Proteosome, TAP loss, β2M loss, Class I loss or ↓ upregulation

Tumor Cell

TCR

CTL

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)

- Loss of antigen presentation capacity by tumor
- 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.
- 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.
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 human tumor (ascites), also in draining lymph node.
A. Generates tolerogenic DC:
   a) “immature”
   b) Producers of tolerogenic cytokines (IL-10, TGF-beta) & IDO (effects tryptophan metabolism)

B. Tolerogenic DC inhibit effector T cell activation
Cancer and Inflammation: Seed and Soil Hypothesis
-- Stromal inflammation as tumor “promoter”
-- ”tolerogenic” healing/remodeling/repair

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Inflammatory stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td>Schistosomiasis</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>H. pylori-induced gastritis</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>H. pylori</td>
</tr>
<tr>
<td>Hepatocellular CA</td>
<td>Hepatitis virus (B and C)</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>HHV8</td>
</tr>
<tr>
<td>Lung CA</td>
<td>Silica, Asbestos</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Asbestos</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Salpingitis/talc/ovulation/endometriosis</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>Barrett’s metaplasia</td>
</tr>
<tr>
<td>Papillary thyroid CA</td>
<td>Thyroiditis</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Prostatitis</td>
</tr>
</tbody>
</table>
Tumor Immunity (Clynes)

Tumor microenvironment
Tumor cells and stromal cells

- Chemokines
  - M-CSF
  - VEGF

  Recruitment

- M-CSF
- VEGF

  Survival

- IL-10
- TGF-β
- IL-4

  Mo Differentiation

Tumor Associated Macrophages/Myeloid Suppressor Cells

- CD11b+/Gr-1+
- monocytes

- iNOS+/Arg+
- Highly reactive peroxynitrite:
  - Can modify tyrosines
  - Induce T cell anergy/apoptosis
  - Chemically modify TCR

RANTES

- IL-6

Tumor cell proliferation/survival
- Growth factors, TNF, IL-1, IL-6
- Polyamines, NO

- MMP, TGF-β
- Chemokines, TF

Matrix remodelling

Adaptive Immunity
- Polarization, Suppression
- Induce T cell anergy/apoptosis
- Chemically modify TCR

Polarized T cells, T reg cells

Progression and Metastasis
- Chemokines, MMP

Angiogenesis
- Chemokines, VEGF, FGF2
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?

**Activating/Inhibitory Co-Stimulation**

<table>
<thead>
<tr>
<th>APC/Tumor</th>
<th>T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>ICOS</td>
</tr>
<tr>
<td>-</td>
<td>CD28</td>
</tr>
<tr>
<td></td>
<td>CTLA-4</td>
</tr>
<tr>
<td></td>
<td>PD-L1</td>
</tr>
<tr>
<td></td>
<td>PD-L2</td>
</tr>
<tr>
<td></td>
<td>PD-1</td>
</tr>
</tbody>
</table>
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 neovascularature, 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 Adoptive T Cell Immunotherapy in Metastatic Melanoma

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.

Proc. Natl. Acad. Sci. USA 101, 14639-14645

Before
1 month after T cell Transfer
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
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 bacilllus; 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.
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).
   The goal is to induce antigen specific effector T cells while eliminating negative immunoregulatory pathways.