Harnessing the power of the immune system to break through cancer-mediated immunosuppression (13-Nov-2004)

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1. Introduction
   a. Paul Ehrlich – the immune system could repress an "overwhelming frequency" of carcinomas
   b. Sir MacFarlane Burnet and Lewis Thomas – "Immunologic surveillance"
   c. Evidence for immune response to cancer
      i. William Coley
         1. Spontaneous tumor regression associated with bacterial infection
         2. Formulated Coley toxins used by he and others to stimulate tumor rejection
      ii. Higher incidence of tumors in mice with B, T and / or NK cell dysfunction / deficiency
      iii. Patients that have tumors infiltrated with lymphocytes often have a better prognosis
      iv. Cancer patients often have a large number of tumor-specific CD8+ lymphocytes in circulation
      v. BCG is used to treat bladder cancer
      vi. Occasional clinical responses to tumor vaccines

2. Innate response to neoplastic cancer - "First line of defense"
   a. Cells express common receptors that recognize evidence of neoplastic transformation
      i. NK cells – NKG2D receptors recognize proteins expressed by a variety of cancers: MICA/B and ULBPs
      ii. NKT cells – T cell receptor (TCR) recognizes phospholipid groups in the context of CD1d molecules on target cells
      iii. γδ T cells:
         1. NKG2D receptors as above
         2. TCR specificity and target unknown
         3. non-specifically activated by bisphosphonate compounds
   b. Rapid response to the appearance of the neoplasm, no selection / expansion of innate immune cells needed
   c. Activated innate cells produce IFN-γ:
      i. Activates dendritic cells to participate in the adaptive immune response
      ii. Promotes leukocyte recruitment to the tumor
      iii. Activates macrophages, other leukocytes to kill tumor cells
      iv. Promotes TH1 differentiation of CD4+ T cells
      v. Promotes MHC class I molecule expression on some tumors

3. The adaptive immune response to cancer – "Antigen-specific immunity"
   a. Types of tumor antigens
      i. Tumor specific antigens (TSA)
         1. Unique to individual tumor (mutated proteins recognized as foreign)
         2. Characteristic of TYPE of tumor
            a. melanoma: MART-1 antigen, tyrosinase
            b. prostate: PSMA
            c. B cell leukemia: CD19, CD20, CD22, CD52
ii. Tumor associated antigens (TAA)
   1. Re-expression of embryonic antigens
      a. Carcinoembryonic antigen
      b. Alpha-fetoprotein
   2. Over-expression of normal "self" antigen

b. Role of antigen-presenting cells
   i. Dendritic cells are most potent antigen presenting cells
   ii. Obtain antigen in the local environment and present to T cells by:
      1. Phagocytosis of dead / apoptotic tumor cells
      2. Receptor-mediated endocytosis:
         a. antigen / antibody complexes
         b. HSP / protein complexes
      3. Pinocytosis of free proteins
      4. Direct binding of peptides to surface MHC molecules
      5. "Nibbling" surface membrane protein from adjacent cells
   iii. Antigen presentation occurs via major histocompatibility (MHC) molecules
      1. MHC class II
         a. Expressed predominantly on antigen presenting cells
         b. "Exogenous" antigens collected from the environment and cleaved into peptides 13 - 18 amino acids in length within lysosome
         c. Presented to CD4 "helper" T cells
      2. MHC class I
         a. Expressed on essentially all cells
         b. "Endogenous" antigens from within cell cleaved by proteasome into peptides 8 - 10 amino acids in length
         c. Presented to CD8 "cytotoxic" T cells
         d. Dendritic cells uniquely "cross-present" exogenous antigen by this pathway
         e. Allows T cells to "see inside" a cell to check for evidence of neoplastic transformation
      3. The MHCI and MHCII molecules and associated peptides engage a T cell receptor (TCR) on the surface of the lymphocyte specific for that peptide
   iv. Provide "second signal" to CD4 and CD8 T cells via co-stimulatory molecules
      1. Positive signal: CD28, ICOS, OX-40, 4-1BB
      2. Negative signal: CTLA-4, PD-1
   v. Produce cytokines to promote differentiation / proliferation of T cells
      1. IL-12 (naïve CD4, CD8 cell): promotes TH1 differentiation
      2. IL-23 (effector CD4, CD8 cell)

c. Role of CD4 cells
   i. Recognize antigen on APCs in the context of MHCII molecules
   ii. Provide positive feedback to APC through CD40L
   iii. Produce cytokines:
      1. Promote expansion / activation of CD8 cells (IL-2, IL-15, IL-21)
      iv. Activate macrophages, NK cells, others to kill tumor (IFN-γ)

d. Role of CD8 cells
   i. Recognize antigen on APCs AND somatic cells in the context of MHCI molecules
   ii. Also express NKG2D receptors
   iii. Produce cytokines: activate macrophages, NK cells, others
   iv. Directly kill target cell: perforins, granzymes

   a. Steps
      i. Elimination
      ii. Equilibrium
      iii. Escape
   b. Tumor must sequentially evade both innate and adaptive responses to become clinically significant
      i. Darwinian selection
   c. By the time the tumor becomes clinically significant, it has already defeated the host’s immune system
5. **Mechanisms of cancer evasion – some examples**
   a. Defective differentiation and function of antigen presenting cells
      i. Immature myeloid cells
         1. Mouse and human tumors produce GMCSF
         2. Stimulates bone marrow to produce immature myeloid cells
         3. Immature myeloid cells populate lymphoid organs and the tumor bed
         4. Prime CD8 cells via MHCI w/o second signal = AINR
         5. Produce oxygen radicals that kill T cells = apoptosis
      ii. Defective maturation / function of dendritic cells
         1. Remain immature in tumor bed
         2. Caused by
            a. Lack of pro-inflammatory stimulus
            b. TGF-β exposure
            c. VEGF exposure
         3. Present antigen to CD4 and CD8 cells, but do not express co-stimulatory molecules
         4. Failure to provide the second signal to T cells
            a. CD4: apoptosis
            b. CD8: antigen-induced non-responsiveness (anergy)
   b. T cell dysfunction
      i. Tolerance to self antigen
      ii. T cell anergy within the tumor microenvironment
      iii. Up-regulation of CTLA-4 on effector T cells, regulatory T cells
      iv. Secretion of immunosuppressive factors within the tumor microenvironment
         1. IL-10
         2. TGF-β
      v. Insufficient CD4+ cell help to support CD8 expansion
      vi. Regulatory CD4+CD25+ T cells
         1. Cell – cell contact
         2. Secrete TGF-β, IL-10
   c. Tumor cell escape mechanisms
      i. Down-regulation / mutation of tumor antigens
      ii. Secretion of soluble NKG2K ligands
      iii. Down-regulation / dysfunction of IFN-γ receptors
      iv. Down-regulation of tumor MHCI-mediated Ag presentation
         1. Decreased MHCI expression
         2. Decreased loading of peptides on MHCI via TAP-1
      v. Tumor expression of molecules that inhibit T cell viability / expansion
         1. B7-H (PDL1, PDL2)
         2. IDO (indoleamine 2,3-dioxygenase)
      vi. Tumor-induced T cell apoptosis
         1. FasL
         2. Galectin-1
      vii. Tumor resistance to NK, CD8+ T cell granzymes, perforin

6. **Strategies to break through tumor immune suppression of the adaptive immune response – some examples**
   a. Goal of immunotherapy – activate the host immune system to recognize and destroy the tumor
   b. Use of monoclonal antibodies to kill tumor cells
      i. Antibody targeted to tumor antigen
      ii. Block growth signals
         1. anti-HER-2/neu
         2. anti-erb-B1: blocks epidermal growth factor receptor
      iii. Complement-mediated lysis
      iv. ADCC
         1. anti-CD20 (Rituximab)
         2. anti-CD52 (CAMPATH)
      v. Antibody conjugates
         1. anti-CD20 °Ytrrium (Zevalin)
2. anti-CD22 Caleachimycin

c. Activate innate immune cells
   i. NK cells: IL-12, IL-2
   ii. NKT cells: IL-2, α-Galactosyl-Ceramide
   iii. γδ T cells: IL-2, bisphosphonate compounds, other

d. Activate / expand antigen presenting cells
   i. Promote maturation of immature myeloid cells (retinoic acid)
   ii. Expand population of DCs available to participate in immune response
      1. FLT3 ligand
      2. GMCSF
   iii. Activate quiescent DCs to express co-stimulatory molecules
      1. Activate Toll-like receptors on DCs
         a. TLR4: LPS
         b. TLR7: imidazoquinolines
         c. TLR9: CpG
         d. other
      2. CD40 agonist
   iv. Load DCs with antigen in vitro and adoptive transfer
   v. Load DCs with antigen in vivo via vaccine
      1. Whole irradiated cells
      2. Crude tumor glycoprotein preparation
      3. Gene-modified tumor cells
      4. Plasmid DNA
      5. Tumor peptides
      6. Altered peptide ligands
      7. Viral gene transfer vectors
      8. Antigen-modified DCs

e. T cells
   i. Stimulate expansion of CD8+ population in vivo
      1. Systemic administration of IL-2, IL-15, IL-21
      2. Local administration of IL-12, IL-23
   ii. Block negative regulatory cytokines in vivo
      1. IL-10
      2. TGF-β
   iii. Block negative regulatory signals on T cells in vivo
      1. anti-CTLA-4
      2. anti-PD-1
   iv. Deplete regulatory T cells
   v. Stimulate positive regulatory signals on T cells in vivo
      1. OX40 agonist antibody
      2. 4-1BB agonist antibody
   vi. Adoptive transfer of in vitro activated CD8+ T cells
      1. Polyclonal
      2. Monoclonal
      3. Lymphoid depletion prior to adoptive transfer allows for "homeostatic expansion" of transferred lymphocytes

7. "Polyimmunotherapy"
   a. Given that clinically relevant neoplasms have already developed the ability to mutate and thwart the host response, it is likely that successful attempts to eradicate the tumor will require that it be attacked simultaneously by several different mechanisms - akin to polychemotherapy.
   b. Stimulate expansion of CD8+ population in vivo following vaccination
      i. Systemic administration of cytokines (IL-2, IL-15, IL-21)
      ii. Local administration of IL-12
      iii. Provides tumor antigen to activate CD8+ T cells AND cytokine to promote CD8+ T cell activation / expansion
   c. TRICOM approach
i. Sequential immunization with recombinant vaccinia virus and defective avipox virus containing the transgenes for carcinoembryonic antigen (CEA) and a triad of T-cell costimulatory molecules (B7-1, ICAM-1, and LFA-3)

ii. Provides tumor antigen to activate CD8+ T cells AND costimulatory molecules to promote CD8+ T expansion

   i. Adoptive transfer of tumor-specific T cells
   ii. Antigen-specific vaccination with altered peptide ligand (rather than native peptide)
   iii. Co-administration of T cell growth and activation factor
   iv. Provides effector cells, antigen AND cytokine to promote CD8+ T cell activation / expansion

8. **Summary and future directions**

### References


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