MEDICAL AND APPLIED IMMUNOLOGY NOTES

MIIM3003

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Contents

Lecture 1 – Immune response to tumours

Traditional pillars of cancer treatment; not curative, slows the growth of cancer:

1. Surgery
2. Radiotherapy
3. Chemotherapy
4. Immunotherapy – very effective therapy in some cancers (modern)

Cancers are derived from own cells (but are also different to our immune system)

Tumours and immune system

Problem – Tumours result from uncontrolled growth of self-tissue, I.S is programmed to be self-tolerant and may not be sufficiently different to be recognised

Evidence – Lymphoid infiltrates found in tumours means better prognosis (normal cells don't infiltrate tissue), in vitro T cell responses to tumour cells and occur more frequently in immunosuppressed patients.

Mechanisms of immune recognition and prevention of cancer

Control of tumorigenic infections (viruses) – cancer caused by viral infections

Epstein-Barr virus – widespread and latent infections in humans, normally controlled but can cause (B cell) lymphoma.

Immunosuppression can increase risk of virally induced cancer; increased relative risk for transplant patient, non-global increase in risk suggests that only some conditions are associated with viruses

Evidence for existence of tumour antigens – mouse previously immunised with a tumour was resistant to the same tumour but not another tumour

Types of tumour antigens – Foreign or mutated

1. **Products of mutated genes** – cancers carry many mutations
   - Mutated oncogenes can lead to cancer; these are altered self-proteins (oncogenic)
   - Variation in the mutation rates differ in different cancers and affects recognition
2. **Products of oncogenic viruses** – Oncogenic virus-derived protein/peptide often expressed by tumour
   - E6/7 proteins from HPV16; EBNA-1 protein from EBV
   - Highly immunogenic, foreign
3. **Altered glycolipid/ glycoprotein antigens** – abnormal forms/levels on surface glycoproteins/glycolipids
   - MUC-1 glycoprotein – altered form expressed by breast carcinoma; some glycolipids are often overexpressed in melanoma
4. **Aberrantly expressed antigens (unmutated)** – Cancers often over-express genes that are silent in most adult tissues (MAGE, MART & NY-EOS1 Ag); usually expressed in the testes (CT antigens)
5. **Tissue specific differentiation antigens** – Tumours often express molecule normally present on cells of origin
   - CD20 – on mature B cells and cancers; CD30 – activated B cells and cancers
   - A-CD20 widely used for B cell leukemia/lymphoma therapy; often in combination with chemotherapy for B cell cancer

Generally, tumour cells can either present antigens novel to the adult immune system or the overexpression of normal self-protein (I.S may be tolerant to only little expression of the protein, but becomes activated in larger quantities)

Adaptive immunity to tumours

CD8+ T cells

- Directly lyse tumours presenting Ag on MHCI
- Major player in anti-tumour responses
- Require CD4 T cell help
- CD8 resident memory T cells implicated in tumour immunity

CD4+ T cells

- Usually indirect role
- Recognise shed Ag/MHCII on APC
- Produce cytokines to help CTL but can also produce tumoricidal cytokines

B cells

- Ab bind to Ag and facilitate a range of responses that lead to tumour death (ADCC, cellular activation via FcR ligation, NK cell killing and complement dependent tumour lysis)

NK cells – activation depends on a balance between stimulatory (FcR, CD2, NKG2D) and inhibitory (MHC-I binding Receptors; KIR, NKG2A Ly49A); *important to catch tumours with down-regulated MHCI*

NKG2D – allow recognition of stressed cells which express stress ligands (MIC-A, RAE-1, ULBP); activate NK cells, even if the target still expresses MHC-I

Unconventional T cells – NKT cells (semi-invariant TCR), MAIT cells (semi-invariant TCR) and γδ T cells; target distinct features of cancers, and can be one-size fits all

NKT cells – express some NK markers (NK1.1) and an αβ TCR; recognise CD1d and glycolipid; produce IFN-γ, IL4, TNF, IL17 that provide help to NK and CTL in vivo; enhance DC activation and maturation; kill tumours in vitro (perforin-dependent)

- Exogenous glycolipid can be used therapeutically to induce rejection of the tumour (α-galactosylceramide
- Reduction in the frequency of NKT cells are important in the progression from a stable to a progressive form

High levels of γδ T infiltrates represents the most favourable outcome in human cancer

Tumour escape mechanism

1. **Low immunogenicity** – not enough difference for the body to recognise; unproductive, ignorant response
2. **Tumour treated as self-antigen** – Ag taken up and presented by APCs in absence of co-stimulation and tolerize T cells
3. **Antigenic modulation** – Unable to eliminate cells that do not have certain immunogenic antigens
4. **Tumour-induced immune suppression** – Factors secreted by tumour cells inhibit T cells directly (PD-1)
5. **Tumour-induced privileged site** – Factors secreted by tumour cells create a physical barrier to the immune system

Phases of tumorigenesis

1. Elimination – when tumours arise, I.S recognises them and kill them
2. Equilibrium – variant tumour cells arise that are more resistant to killing, over time a variety of different tumour variants develop
3. Escape – eventually one variant escapes the killing and spreads unchallenged

Persistence of tumour – latent cancer can be transferred from organ donor to recipient (following transplant); development of metastatic melanomas can occur in immunosuppressed recipients in a few months

Lecture 2 – Tumour Immunotherapy

Origins of tumour immunotherapy – William Coley

- Noticed cancer remission occurred sometimes after bacterial infection; and injected bacterial extracts into tumours leading to sporadic tumour rejections
- Enhance immune response via TLR ligation (adjuvant effect)
- BCG used to treat bladder cancer

Current immunotherapeutic approaches to cancer

1. **Preventative immunotherapy –** antiviral vaccines (effective at precenting some viruses, and the cancers that they can induce)
2. **Antibody based immunotherapy** – mAb can be generated against tumour antigens; injected to target, inhibit and kill tumours; either alone or conjugated
    - **mAb alone** – block molecules important for tumour growth or activate FcR+ cells
    - **mAb-toxin conjugate** – deliver toxin straight to tumour cell
    - **mAb-radioisotope conjugate** – take isotope to tumour cell, targets an area of cells

Challenges:

1) **Specificity of Ab**
2) **Penetration into large tumours**
3) **Instability of tumours (escape mutants)**
4) **Limited knowledge of dominant tumour antigens**

Examples of antibody-based immunotherapy

- a-HER2 (Trastuzumab) blocks EGF drive tumour growth (cell cycle arrest)
- a-CD30 (Brentuximab); drug conjugate that targets B cells specifically
- a-CD20 (Rituximab); targets and kills B cells

3. **Cytokine based therapy** – use of cytokines to modify the immune response
    a. IL2 – melanoma, renal, colon; T and NK activation
    b. **IFN**α – melanoma, lymphoma, renal; NK activation
    c. TNF – melanoma, sarcoma; MO and lymphocyte activation
    d. IL12 – melanoma; NK and CTL activation

    Problems
    - Limited success with some tumour types
    - Toxic when used systemically (severe side effects); normally are only restricted to local effects
    - Short half-life; expensive and inefficient
4. **Immunisation with tumour cells or antigens** – patient immunised with killed tumour cells or purified Ag with adjuvant
    - Works in animal models, and better prophylactically than therapeutically. Improved adjuvants and delivery can help this
    - Dominant Ag underrepresented in whole tumours or not be patient specific
    - MHC-restricted Ag presentation
    - Antigenic modulation and risk of autoimmunity

    Examples

    - **B7 transfected tumour cells** – activate tumour specific T cells
    - **GMCSF-transfected tumour cells** – induce DC development at tumour site; these DC migrate and then present tumour Ag to immune system
5. **Adoptive cell therapy**

    Tumour infiltrating Lymphocytes – isolated from biopsies, expand in culture with IL2 and TCR stimulation, enriched for tumour specific CTL; *Good response*

    Adoptive CAR T cell therapy – Patient T cells engineered to express modified Ag receptors fused with

intracytoplasmic T cell stimulatory and costimulatory domains. Expanded in vitro and reintroduced in patient

3rd generation – CD28, 4-1BB with Ag recognition domain – *very good* response

6. **Checkpoint blockade inhibitors** – blocking immune inhibitory molecules
    a. a-CTLA-4 (ipilumumab) – prevents the ligation of CTLA-4 with B7 to prevent activation
    b. **PD-1 (Nivolumab)** – prevents the ligation of PD-1 with PD-L1

Combined therapy has more promising results, through the side effects are also greater

Lecture 3 – Strategies to enhance the immune system against cancer

Using the immune system against cancer

- The immune system is potentially a high specific and effective weapon against cancer
- An effective immune response requires the expansion and activation of tumour-specific T cells

Treatment of established tumour in mice with cultured lymphocytes

- Lymphokine active killed cells and IL-2 could inhibit 3-day lung metastases of methylcholanthrene induced tumours
- TIL and IL-2 could eradicate established metastases

Although TIL therapy is effective, cannot generate endogenous tumour-reactive lymphocytes from all patients (most malignancies)

- Most tumours are non-immunogenic, difficult to isolate lymphocytes
- Cancers can down regulate MHCI

CART cell therapy

Advantages – Non-MHC restricted, specific for recognising tumour associated antigens

Vector – Retroviruses as gene vehicles; co-culture retroviruses with packaging cell line with enriched naïve T cells; separation and analysis of receptor expression and assays

Shown to reduce established tumour burden, bind more effectively and kill faster than T cells

CART cells targeting the Lewis-Y tumour-associated Ag

Le^Y – fucosylated carbohydrate on variety proteins and lipids

Overexpression in carcinomas, AML and myelomas; expression associated with poor prognosis and normally expressed in different levels in different cells. Humanized Ab and scFv available

Clinical protocol

Primary – safety

Secondary – cell persistence and trafficking, serum cytokines, anti-cancer activity

Preconditioning with fludarabine and 10% of cells labelled with ^{111}Indium; showed the movement of CAR T cells into the bone marrow, blood and skin (different parts of the body and effector sites)

CART cells targeting Lewis Y Ag well tolerated in all AML patients; biological responses were observed in AML patients following CART cell therapy; persist for a long time to target sites

Checkpoint blockade therapy – a mechanism of immune system control that cancer cells take advantage of to avoid immune response. Highly expressed on cells in the tumour microenvironment.

Lymphatic tissue – mainly CTLA-4 involved; leads to diffuse and non-specific T cell activation

Peripheral tissue – mainly PD-1; leads to more restricted spectrum of T cell activation

Response showed that they were not good for cold cancers (non-inflamed)

Combined therapy

- Enhanced immune activity in patients compared to either therapy alone
- a-CTLA4 enhance T cell priming and activation of T cells and can deplete tumour microenvironment of Treg
- a-PD1 can removed the inhibition of cancer cell killing
- Immune-related toxicities were increased

Combination of CAR T cells and a-PD1 enhances regression of established breast cancer

Other immunosuppressive pathways

CD73/adenosine – produced in large numbers inhibits proliferation of T cells, decreased cytotoxicity of NK cells, and decreased IL-12 and TNFα and increased IL-10 in MO

Generated through ATP use

Lecture 4 – Killers, Vaccines and Immunity

Vaccine challenge:

1. Misinformation, empowerment and failure to convince people of relative risk
2. Affordability
3. Economics and prevalence

Infectious disease has shaped the history and development of humans, modern human lifestyle factors facilitate the emergence and spread of new infections, immune responses to infection can protect or kill