Monoclonal Antibodies

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Monoclonal antibodies (MAbs)

- Polyclonal antibody responses (summary).
- Benefits and limitations of polyclonal antibody responses.
- Short review of antibody assay methods.
- Concepts and derivation of MAbs.
- Assays for MAbs - sensitivity c.f. polyclonal antibodies.
- Mabs in clinical/veterinary diagnosis/therapy.
- Problems of rejection/immune stimulation by Mabs in vivo.
- Concepts and derivation of MAbs from heterohybridomas.
- Applications of heterohybridomas in veterinary species.
- Future prospects for MAbs and heterohybridomas.
- Summary, Acknowledgements, Resources and Literature.
Polyclonal antibodies: production

- Immunise host animals with purified antigen (with adjuvant) - approx 2-4 weekly.
- Test sera and select animals with high reactivity.
- Re-boost and collect large volume of blood.
- Prepare serum and isolate immunoglobulins.
- Re-immunisation and bleeding are possible.
Polyclonal antibodies: limitations

- Life span of host animal(s).
- Considerable individual variation: (select a “favourite” - research progress dependent upon life and health of (say) one rabbit).
- Desired antibody is only a minor component of serum (contains “natural”, cross-reactive and contaminating antibodies).
- “Specific” antibodies are heterogeneous mixtures (varying concentrations/ affinities/activity).
- Solution: affinity purification of Igs with purified antigen.
- Result: limited supply of required antibody.
Some properties of serum polyclonal antibodies

- Total serum volume from rabbit ~ 250ml
- Protein concentration ~ 70 mg/ml
- All immunoglobulins ~ 15 mg/ml
- Specific antibody ~ 100 µg/ml
- Max available specific antibody ~ 25mg
Some commonly-used assays for antibody

- **ELISA** (enzyme-linked immunosorbent assay)
- **RIA** (radioimmunoassay)
- **Gel Precipitation** (radial, semi-quantitative)
- **Fluorescence-based** (microscopic, cells and tissue sections)
- **Flow cytometry** (e.g. FACS analysis, quantification of specific cells)
- **Haemagglutination** (e.g. influenza)
- **Complement fixation** (e.g. herpesviruses)
- **Virus neutralisation**
Summary of some assay methods
How can we make large amounts of antigen-specific antibody?

- Serum contains Ab produced by millions of B cells, only 1% of total Ab is “specific”.
- Isolate individual B cells?
- Select and culture/expand individual B cells?
- Clone individual B cells?

**Requirement:**

Immortalised B cells continuously secreting their specific Ab
Monoclonal antibodies: concepts

- Spontaneous somatic cell hybridisation sometimes observed *in vitro* (1960’s)
- Some viruses shown to increase hybridisation frequency (e.g. Sendai)
- Fusions of human and mouse tumour cells (1960’s)
- Hybrid cell lines shown to express genes from both parental cells (1963)
- Selections of parental cells for drug resistance allowed only hybrids to survive (1964)
Monoclonal antibodies: concepts/methods

Hybridoma selection based on drug resistance

- Identify immortalised mouse myeloma cell line (e.g. NS1 myeloma derived from BALB/c mice)
- Mutate to make deficient in enzymes necessary for DNA synthesis (HGPRT and TK)

HGPRT = hypoxanthine-guanine phosphoribosyltransferase
TK = thymidine kinase
### Biosynthetic mutations in mutant myeloma cells

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Present</th>
<th>Absent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGPRT</td>
<td>✗</td>
<td></td>
<td>Can’t use hypoxanthine</td>
</tr>
<tr>
<td>TK</td>
<td>✗</td>
<td></td>
<td>Can’t use thymidine</td>
</tr>
<tr>
<td>Rescue path</td>
<td>✔️</td>
<td></td>
<td>Can use Uridine</td>
</tr>
</tbody>
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**BUT: Culture medium contains HAT which blocks the Rescue Pathway**

H = hypoxanthine
A = aminopterin
T = thymidine
Monoclonal antibodies: concepts/methods

Hybridoma selection based on drug resistance

- Mutated myeloma cells will not grow in presence of aminopterin.
- Fuse myeloma cells to normal B cells from immunised mouse (PEG used routinely now)
- Unfused myeloma cells die (aminopterin)
- Unfused normal B cells die after 1-2 weeks anyway
- Only hybridomas survive (they can use hypoxanthine and thymidine).
Production of monoclonal antibodies

1. Mouse immunized with antigen
2. Spleen
3. Cells
4. Myeloma cells
5. Cell fusion in polyethylene glycol
6. Selection of hybrids in HAT medium
7. Cloning
8. Assay for antibody production
9. Identify positive clones
10. Recombine positive hybrids
11. Freeze cells for storage
12. Monoclonal antibodies
Critical steps

Antigen need not be pure

2-4 weeks TLC

“Feeder” cells required

Must have a specific screening method!

1° screen of supts for Mab activity

Re-screen often

e.g. Ascites
Advantages of monoclonal antibodies

- Standard/routine procedure now
- Impure antigens may be used - needs specific selection and screening strategy
- Can select Mabs with specific biological effects or reactivity for specific structures
- Unlimited supply of homogeneously reactive antibodies
Application of monoclonal antibodies

- Purification of molecules/viruses etc:
  - Couple Mab to solid surface and use to affinity purify the molecule of interest (several-fold in one step, Coligan et al 1997, Current Protocols in Immunology, J Wiley & Sons)

- Sensitive detection assays:
  - Used to detect autoantigens, viruses, bacteria, body fluid components

- Detection of cell surface markers:
  - CD markers, HLA molecules, cytokines and receptors (Winkelstein & Donnenberg 1997, Clinical application of flow cytometry, CRC Press)
Application of monoclonal antibodies

- **Applied chemistry:**
  - Make Mabs against enzyme inhibitors - potential to serve as enzyme and show catalytic function (Schultz & Lerner 1995, Science 269, 1835-1842)

- **Gene identification:**
  - Make Mabs against portion of predicted protein encoded by a gene and use to look for gene expression and function
Disease therapy:

Mabs against cytokines and activated cell surface markers used to treat autoimmunity and transplant rejection (Moller 1996, Immunol. Rev. 129, 1-201)

Anti-TNF Mabs used to treat RA (infliximab, Vitella et al 1993, Immunol. Today 14, 252-259). Couple Mabs to toxins/drugs for cancer therapy (magic bullets)

“Humanised” Mabs (replace mouse Ig structures with human counterparts)

NB: “Antibodies as therapeutic entities” and “Engineering antibodies for therapy” to be delivered as part of this module Spring 2004.
IL4, IL5, IL6, TGF-ββ
IL10
IFNγ
Th1 inhibits production
inhibits proliferation
Th2
IL4, IL5, IL6, TGF-β
IL10
Macrophage activation
Mast cell
B cell
Eosinophil
Antibody
Th1 inhibits production
Th2

Disease Therapy with MAbs

Monoclonal Antibody Treatment for Systemic Lupus Erythematosus

This study is currently recruiting patients.
Sponsored by: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

This study will examine the safety and effects of the monoclonal antibody MRA in patients with systemic lupus erythematosus (SLE). Antibodies normally fight invading organisms. In autoimmune diseases, such as lupus, however, antibodies attack the body's own tissues. MRA is an antibody manufactured in the laboratory that blocks the action of interleukin-6 (IL-6), a substance that increases antibody production and is involved in inflammation that may cause organ damage in SLE.

One hypothesis as to how these antibodies work, in simplistic terms, is that the antibody attaches to the antigen and then through its FC portion interacts with NK cells or the cellular effector mechanisms of the immune system. An alternative view is that when the antibody attaches to the antigen, it induces the complement cascade and ultimate cell death through that mechanism.

Rituximab is an antibody to take advantage of the technology to make chimeric or humanized antibodies. It is chimeric with the variable region being murine and the backbone human IgG1. It targets CD20, which is expressed on all B-cells, but not T-cells, or NK cells, and is involved in regulating the cell cycle.

Another antibody is Campath-1H, which targets CD52 expressed on B-cells and T-cells and monocytes, and is ubiquitous throughout the human population. When the humanized version of Campath was given to patients with a variety of lymphoid malignancies, it was found to be very effective at depleting circulating tumor cells in the blood and bone marrow.

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Fender Telecaster Custom 1973
**In vitro methods for MAb production**

- Cloned hybridomas were usually propagated as ascites in peritoneal cavity of BALB/c mice (pain and distress).
- Ethical Review Process and Home Office regulations require attention to 3Rs.
- Ascites banned in UK now, unless specifically approved by HO.
- *In vitro* systems can generate about 100ml Mab at ~1mg/ml (see supplied list)
In vitro methods for MAb production

i-Mab gas-permeable bag
In vitro methods for MAb production

CELLine CL 6 Culture System
In vitro methods for MAb production

Metabolites
Nutrients
Oxygen

Cell products
Cell mass

Nutrient supply chamber
Oxygen supply chamber

CELLline culture system

Nutrient Membrane
Gas Membrane
In vitro methods for MAb production

CELLline culture system
In vitro methods for MAb production

Tecnomouse culture system
Fender Telecaster Custom 1973
Developments in species-specific monoclonal antibodies

- Very few human myeloma cell lines
- Even less myeloma cell lines in other species
- Inter-species fusions are possible but give low hybridisation frequency and unstable hybrids

**Possible Solution:**

Use heterohybridoma cell lines as fusion partners
Respiratory Diseases Seminar Room
Newmarket
Species-specific Mabs: basic method

- e.g. Consider the horse
  - Fuse mouse myeloma cells to normal horse lymphocytes.
  - Select and clone immortal (non-antibody secreting) hybrids.
  - Use as fusion partners for B cells from horse immunised against influenza virus.
  - Resultant Mabs are “horse”, not mouse.
The production and application of non-roden monoclonal antibodies in veterinary science.

Groves DJ, Tucker EM.

Department of Biochemistry, University of Surrey, Guildford, Great Britain.

The requirement for monoclonal antibodies derived from species other than rats and mice is becoming increasingly realised in veterinary, as well as human, medicine. This paper reviews current knowledge of the production of inter-species hybridomas (heterohybridomas) by the fusion of rodent myeloma cell lines with lymphocytes from species of veterinary importance. To date a number of monoclonal immunoglobulins derived from sheep, cattle, pig, rabbit, mink and primate species have been produced to a variety of different bacterial, viral and nematode pathogens as well as to blood group and MHC determinants and to hormones. The technique opens up a number of possibilities for the future; some of these applications are discussed in relation to the antibodies produced thus far.
Species-specific Mabs: basic method

e.g. Consider the horse

Antibodies produced from:

(Horse x Mouse) x Horse Heterohybridomas
Not a BALB/c mouse
Scheme for making horse/mouse heterohybridomas (immunised horses)
Equine-influenza-specific equine monoclonal antibodies


The production of equine monoclonal immunoglobulins by horse-mouse heterohybridomas.

Richards CM, Aucken HA, Tucker EM, Hannant D, Mumford JA, Powell JR.

AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, UK.

Studies were carried out to determine the optimum conditions for the production of equine monoclonal antibodies (MAbs). Lymphocytes from ponies immunised with influenza A equine 2 virus, isolate A/Equine/Newmarket/79 (H3N8) were fused with mouse myeloma (NSO) cells and with horse-mouse heterohybridomas made aminopterin-sensitive by selective growth in 8-azaguanine. Although all fusions initially resulted in heterohybridoma colonies that secreted equine immunoglobulin, many of these were unable to maintain secretion for longer than a few weeks. Increasing the time between immunisation and the booster injection of Newmarket/79 virus, the inclusion of Freund's incomplete adjuvant and the use of an aminopterin-sensitive primary heterohybridoma as the fusion partner, improved the production of HIg-secreting heterohybridomas. After two clonings eight cell lines were established which maintained anti-Newmarket/79 antibody secretion for over a year. FACS analysis of the cell lines provided a useful means of predicting breakdown of MAb secretion by the cell lines, thus enabling re-cloning to be carried out in time.
Acknowledgements

Wellcome Trust
Home of Rest for Horses
Horserace Betting Levy Board
European Union
Centaur Inc
Animal Health Trust
BBSRC Babraham

Betty Tucker
Claire Richards
Julia Kydd
Zoe Swann
Ken Smith
Key references listed in slides

Commercially available *in vitro* culture systems for Mabs

Homepage and Equine Leucocyte Antigen Workshops: www.vetmed.wisc.edu/research/eirh

Equine Immunology Mailbase Server: equine-immunology@mailbase.ac.uk

A MOTTO FOR A HAPPY LIFE

- Work as if you don’t need the money
- Love as if you’ve never been hurt
- Dance as if no-one is watching
Animal Health Trust  Newmarket

- Lanwades Hall
- MRI Scanner
- Visitors Centre
- CPM
- CSAS
- CES