

Immunogen vs Antigen

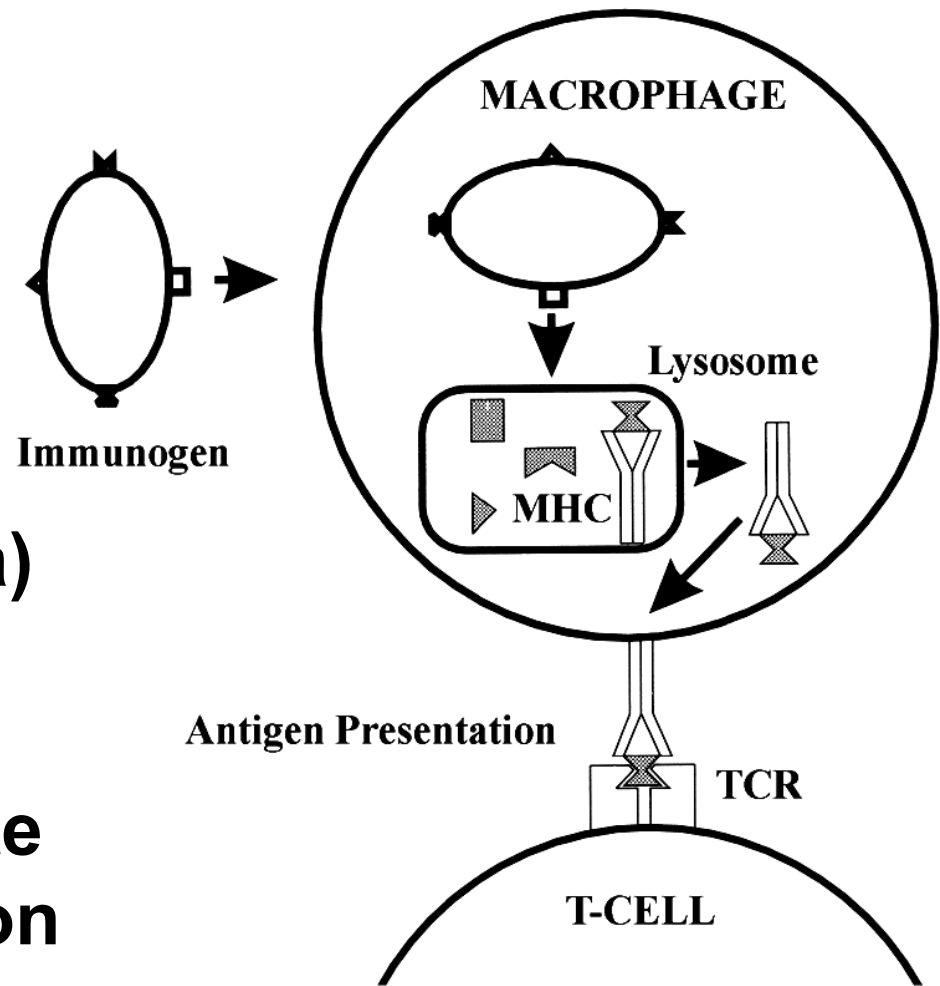
- immunogen induces immune response
- antigen reacts with products of the immune response
- immunogenicity
 - nature of the immunogen
 - ability of immune system to react
 - possible to manipulate

epitope (antigenic determinant)

- recognized by antibody
- recognized by T-cell receptor

Features of Immunogens

- foreignness
- molecular complexity
- molecular size (>10 kDa)
- B-cell epitope
- T-cell epitope
- MHC class II binding site
- degradation/presentation
- particulate/phagocytosis



B-cell epitopes 4-8 hydrophilic residues
(linear or conformation)

T-cell epitopes 8-15 amphipathic residues

Problems Associated with Immunogens

Property	Defect	Result	Remedy
Lacks B-cell epitope	No B-cell recognition	No response	None
Lacks Class II site	No presentation	No response or only primary response	Conjugate with class II site or switch animals
Lacks T-cell epitope	No T-helper involvement	No response or only primary response	Conjugate with T-epitope or switch animals
Non-degradable	No presentation	No response or only primary response	None
Small size	No T-helper involvement	No response	Conjugate with carrier (hapten)
Non-particulate	Poor phagocytosis	Weak response	Self-polymerize or couple to beads

Preparation of Immunogens

- need sufficient quantities of highly purified protein
 - relative immunogenicity
 - choice of animal
 - adjuvant

	Native	vs	Denatured
• conformation			
• SDS-PAGE	conformational epitopes		sequential epitopes
	more immunogenic		more highly purified

Adjuvants

- enhance immune response
- generally 2 components:
 - 'depot effect'
 - immunomodulation

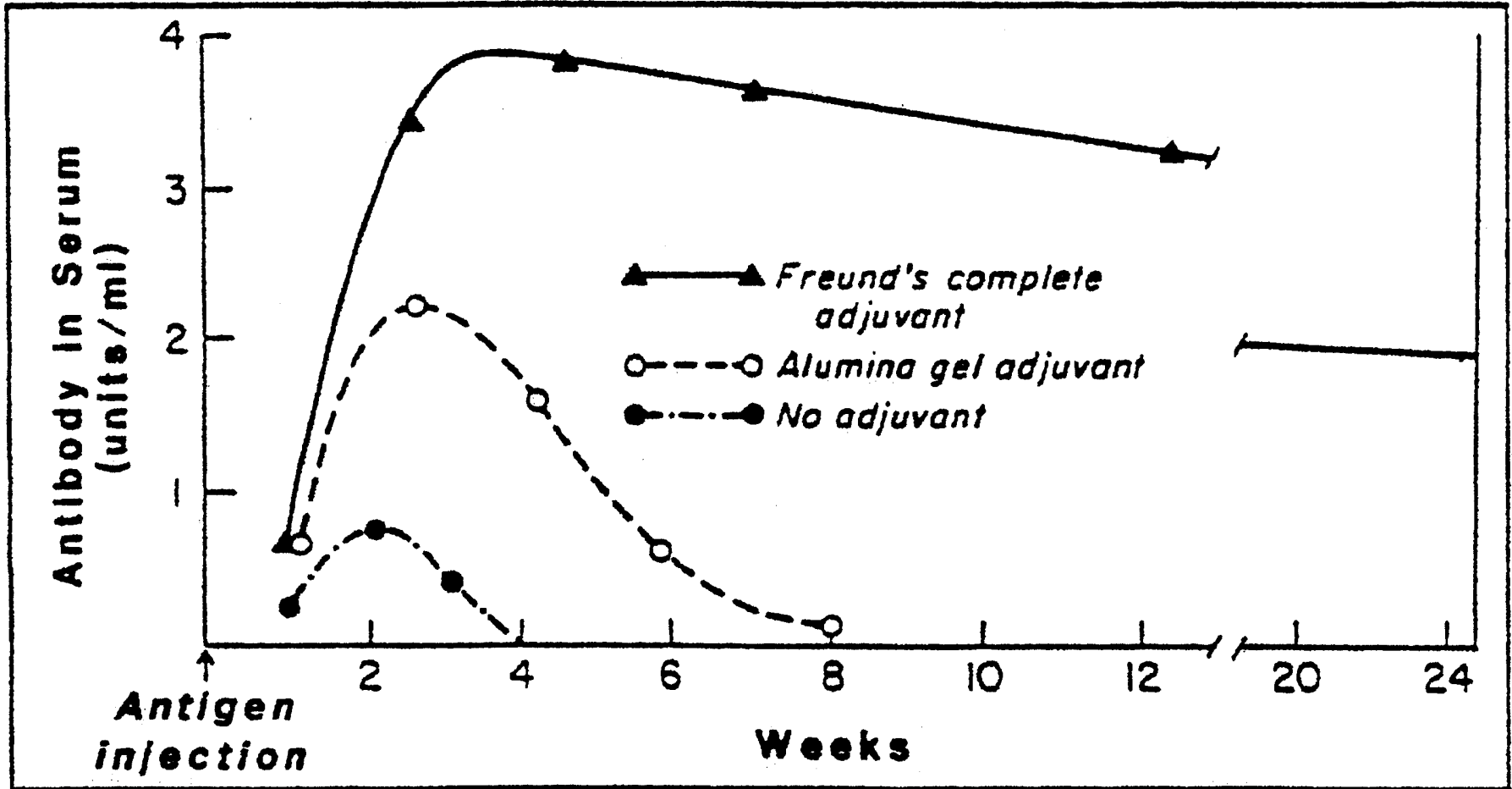


- killed mycobacteria
- muramyl dipeptide
- lipopolysaccharide

Major Classes

- aluminum salts
- water-in-oil emulsions
- oil-in-water emulsions
- saponin
- liposomes

Augmentation of Antibody Formation with Adjuvants



Choice of Animals

Animal	Advantages	Disadvantages
Rabbits	<ul style="list-style-type: none">• large amounts of sera• strong antibody response• easy to maintain	<ul style="list-style-type: none">• require more immunogen• high background
Mice/Rats	<ul style="list-style-type: none">• monoclonal antibodies• inbred strains• easy to maintain	<ul style="list-style-type: none">• small amounts of sera
Guinea pigs	<ul style="list-style-type: none">• generally high titer antibodies• easy to maintain	<ul style="list-style-type: none">• difficult to bleed• small amount of sera
Goats/ Sheep	<ul style="list-style-type: none">• very large amounts of sera	<ul style="list-style-type: none">• difficult to maintain
Chickens	<ul style="list-style-type: none">• good for some highly conserved mammalian antigens	<ul style="list-style-type: none">• difficult to maintain

Injection Routes and Doses

Route		Comments
subcutaneous	sc	easy injections
intramuscular	im	slow release
intradermal	id	difficult injections, slow release
intravenous	iv	only for boosting, no adjuvants
intraperitoneal	ip	easy injections, common in mice

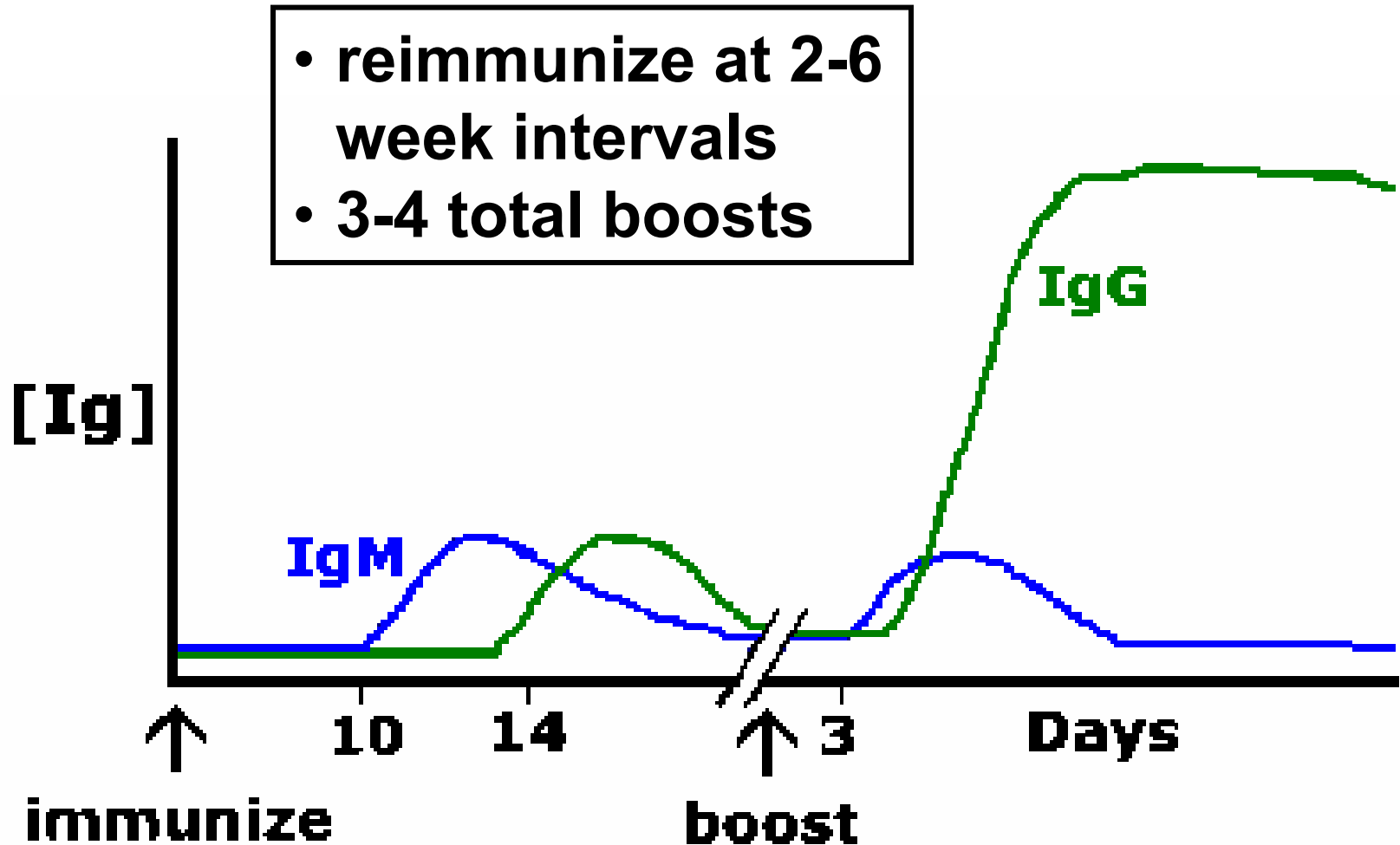
Dosing Factors

- immunogenicity
- purity
- adjuvant
- animal

rabbits	0.05-1 mg
mice	5-50 μ g

Boosting

- reimmunize at 2-6 week intervals
- 3-4 total boosts



Processing Blood

- **plasma: collect with anti-coagulants, remove cells (ie, centrifugation)**
- **serum: allow blood to clot (preferable)**
- **± inactivate complement (heat 56°, 30 min)**
- **enrich IgG**
 - **ammonium sulfate ppt. (40-50% sat.)**
 - **anion exchange chromatography**
 - **affinity purify**
 - **IgG in general (IgG binding proteins from bacteria)**
 - **specific epitope (or pre-absorb with unwanted epitopes to lower background)**