UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION
INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
I.C.T.P., P.O. BOX 586, 34100 TRIESTE, ITALY, CABLE: CENTRATOM TRIESTE

SMR.940 - 36

THIRD AUTUMN WORKSHOP ON MATHEMATICAL ECOLOGY

(14 October - 1 November 1996)

"Immunodominance, competition and evolution in immunological responses to helminth parasite antigens"

Daren J. Austin
Department of Zoology
University of Oxford
Oxford OX1 3PS
United Kingdom

These are preliminary lecture notes, intended only for distribution to participants.

Immunodominance, competition and evolution in immunological responses to helminth parasite antigens

Daren J. Austin

Wellcome Centre for the Epidemiology of Infectious Diseases,
University of Oxford, South Parks Road, Oxford OX1 3PS.

24 October 1996

§1. Introduction

1) Brief outline of helminth infections focusing on Schistosomiasis (focus for Vaccines).

2) How we can model the immune response to helminth infection using very simple mathematical models.

3) Show how Introducing more biological assumptions such as genetic variation of antigen epitopes provides possible evolutionary strategies.

4) Finally discuss some predictions of the model and how these can be tested both in the laboratory and the field.

§2. Helminth Infections

1) 70% of the global population live in the developing world, where helminths are one of the commonest infections and 1/3 of the population harbours intestinal helminths such as Ascaris lumbricoides and Trichuris trichuria.

2) 200m people are believed to be infected with the Schistosome fluke *Schistosoma haematobium*.

3) Children born in areas where helminth infections are endemic can expect to harbour worms for most of their lives owing to repeated exposure to infection and inability to form lasting immunity.

§3. Schistosomiasis

1) In the case of Schistosomiasis, infection is via contact with fresh water infected with free swimming cercariae released from snail intermediate hosts.

2) After penetrating the skin they transform into migrating schistosomulum larvae. During this stage the parasite appears most vulnerable to immune attack.

3) Schistosomulum migrate through the bloodstream to the hepatic portal system where male and female worms differentiate, pair and migrate into the intestine or bladder.

More...

4) After 6-12 weeks females begin laying between 300-3000 eggs per day.

5) Adult worms can survive within the host for 3-8 years despite abundant evidence of immunological recognition.

6) This survival bears testimony to the effective immune avoidance strategies helminths have evolved to enhance their reproductive fitness.

§4. Vaccine Development

1) The development of an effective vaccine against major helminth infections has proved an elusive goal, although significant progress is now being made.

   a. Chemotherapy remains the intervention of choice being both effective and inexpensive.

   b. Problems of reinfection means frequent treatments are necessary.

2) A vaccine could in principle overcome the problems of reinfection by reducing transmission levels within the community.

3) In the case of *Schistosoma mansoni,* partial immunity in rodent models can be transferred by immune sera and monoclonal antibodies against a variety of schistosome antigens.

4) Some of the major B and T cell epitopes have been mapped leading to the possibility of constructing synthetic vaccines

5) Using Triose-Phosphate Isomerase antigen, Multiple Antigen Peptides (MAPs) have been recognised in more than one strain of mouse.

6) Interpretation of the efficacy of synthetic antigen will depend on an understanding of the underlying dynamics of the host immunological responses to antigens that present multiple epitopes to the immune system

§5. Field Studies

1) Intensity of infection rises during childhood to a peak and declines in adults who show lower levels of reinfection

2) Is this pattern a result of Exposure or Acquired immunity?

3) Treating two comparable populations, and studying reinfection rates shows a 1000-fold reduction in reinfection in adults. A much greater reduction than exposure alone.

4) No lasting immunity is present in children due to the action of IgG and IgM blocking antibodies.

5) Levels of protective IgE antibody increase throughout childhood and saturate in adults.

§6. Age-Related Intensity Profile of Schistosomiasis Infection

1) Data collected from Kisumu in Kenya by Mahmoud and Warren (Clin. Res. **28,** 474).

2) Intensity of infection measured by egg counts per gramme faeces.

3) Characteristic increase in intensity which peaks at ages 10-15 and falls away in adults.

4) Little difference between males and females (who have slightly reduced exposure).

§7. Immune Response

1) Individual mechanisms rarely act in isolation and any resistance is likely to be effected by a multicomponent response.

2) Prime candidates are the humoral and cellular components most evident during infection: Elevated IgE levels and eosinophilia.

3) Two subsets of differentiated T helper cells: Th1 and Th2 characterised by their cytokine profiles are believed to be cross-inhibitory:

   a. Th1 response is protective,

   b. Th2 response is associated with chronic infection.

4) Cross- inhibitory effects result in a characteristic imbalanced Th1/Th2 ratio.

§8. Modelling the Immune System

1) Population dynamics is well established for modelling the epidemiology of infectious diseases.

2) The immune system is highly dynamic and involves large populations of different classes of cells, often interacting in a complex manner.

3) We would like to capture some aspects of this action using mathematical models.

§9. Modelling Aims

1) Aim to capture the essence of the parasite-host immunological defenses:

   a. Antigens are secreted, excreted and surface molecules,

   b. T cells proliferate in response to antigen,

   c. each antigen has a number of epitopes,

   d. epitopes are subject to genetic variation.

§10. Modelling Assumptions

1) Individuals are exposed to infection at a **constant** rate determined by their environment. This may be via
   a. contact with water (Schistosomes),
   b. grass (Hookworm),
   c. or ingestion (Ascaris).
2) No discrimination between T and B cell components.
3) No discrimination between T cell subsets.
4) T cells undergo clonal expansion on contact with antigen or via the presence of Antigen Presenting Cells (APCs)

§11. Modelling Assumptions 2

5) Abundance of Antigen Presenting Cells is related to parasite abundance. **More** parasite means **more** antigen
6) Abundance of T cells is proportional to the effector arm of the immune system. **More** T cells means **greater** immune response
7) Immune system reduces parasite life expectancy not parasite establishment via a killing process.
8) **Criticisms**
   a. Criticism of modelling usually centres on the fact that the models are often to simplistic and neglect much of the Biology. **Throwing the Biology out with the Bathwater!**
   b. Hopefully this talk will convince you that using just these simple assumptions, reasonable conclusions can be made about the activity of the immune system.

§12. Basic Model of a Cellular Response to Helminth Antigens

1) Purple antigen or APC population p(t) bearing a square red epitope.
2) Red T cell population x(t) act solely at this epitope to reduce parasite abundance by some killing mechanism proportional to the population of cells.
3) How can we convert this simple model into equations?

At the risk of losing half the audience,...

§13. Basic Model

1) Λ = Exposure rate (rate at which new parasites enter the host).
   a. For viruses exposure is dependent on viral load because each virion can undergo reproduction at some rate r.
2) μ = natural death rate of parasite ($1/\mu$ = parasite life expectancy).
3) h x(t) = parasite mortality due to immune attack.
4) c = immunogenicity = rate at which **a single cell** will proliferate when exposed to a **unit** quantity of antigen.
5) c p(t) = proliferation rate of T cells in response to antigen.
6) The **more** immunogenic the epitope, the higher c and **faster** proliferation.
7) b = T cell death rate (1/b = T cell life expectancy).
8) What can we say from this model?
   a. If **no** immune recognition then x(t) = 0 and the parasite intensity will saturate to some level where exposure is just compensated by death (Λ/μ).
   b. All parameters except possibly immunogenicity can be measured either in the field or in the laboratory.

Questions?

§14. Numerical Evolution of the Basic Model (Parasite Abundance)

1) Three numerical solutions of p(t)
2) Infection at birth (t=0)
3) Age = Time
4) c = 0.1 shows saturation to $p^* = \Lambda/\mu$ and no immunological recognition
5) Increasing c reduces;
   a. Age of peak parasite abundance.
   b. Adult parasite abundance.
6) parasite is never cleared

§15. Age-related Intensity Profiles

1) The graph on the left shows the earlier *Schistosoma mansoni* data and the one on the right shows *Trichuris* infection.
2) In both graphs there is considerable evidence of acquired immunity: Witness the sharp decline soon after peak intensity.

§16. Numerical Evolution of the Basic Model (Immune Response)

1) Shows the immune response x(t) for the three values of immunogenicity.

2) c = 0.1 shows no immune recognition as expected.

3) Little recognition before t = 5 years old.

4) Increasing c causes immunoactivation and saturation of intensity.

§17. Summary of Basic Model

1) No immune recognition leads to saturation of parasite abundance $\mathbf{p(t)} = \Lambda/\mu$.

2) Immune recognition is only possible if $\mathbf{c > b/p^*}$.

3) Immunogenicity affects age of peak parasite intensity.

4) **Evolution?** We can focus on the selection pressures present:

   a. **Parasite is never cleared due to repeated exposure** and short-lived T cells failing to generate lasting immunity

   b. Host tries to suppress parasite abundance either by **raising c** or increasing T cell life expectancy (**reducing b**).

   c. It is in the interests of the host to mount an effective response as quickly as possible.

   d. The parasite will try and increase abundance by **lowering c** in an attempt to avoid attack.

Questions on Basic Model?

§18. More Realistic Models

1) To counter the charge of over-simplicity we can now introduce more realism into the model in the form of

   a. Multiple epitopes,

   b. Genetic mutation of epitopes.

2) By doing this we hope to be able to see possible evolutionary strategies helminths can use to evade the full immune repertoire.

§19. Parasite Antigens with Multiple Epitopes

1) The simplest extension to the basic single-epitope model is to add more epitopes to the antigen in question, each with its own set of T cells.

More...

2) In this example we see three epitopes being presented to the immune system; Red, Green and Yellow.

3) How can we convert this into a model?

Another half of the audience disappears!

§20. Equations!

1) Notice that each population of cells has its own equation.

2) We have rescaled the model so that P(t) now measures the fraction of the saturated value.

3) All terms are still present

4) $\beta 1$, $\beta 2$ **and** $\beta 3$ **measure immunogenicity**. Now $\beta > 1$ for immune recognition.

5) Red T cells are only stimulated by parasite antigen **not** other T cells.

6) How does the model behave?

§21. Immunodominance of One Epitope

1) In this example **all** three epitopes are sufficiently immunogenic to cause immune recognition, but only one has been seen!

2) Both Red and Yellow epitopes have failed to recognise the antigen.

3) This phenomenon is Immunodominance.

4) Why might this be? Well, looking at the parasite abundance gives us a good idea:

   a. The immune system is trying to **minimise** parasite abundance and it can do so by selecting the **most** immunogenic (Green) epitope.

   b. All three sets of T cells are competing for the same resource (antigen). **Green** has a competitive advantage (faster proliferation) so wins via **competitive exclusion**. (Well known process in Ecology)

5) Immunodominance has been shown in viral infections.

6) Rodent models of helminth infection suggest only that one epitope may be recognised much more than others in a given mouse strain.

7) **The model shows how the immune system can use competition to minimise the intensity of infection.**

§22. Genetic Variation at a Single Cross-Reactive Epitope

1) Having seen the effects of adding epitopes, so let's now look at the effects of introducing genetic variation.

More...

2) In this model a red epitope has **four** variants, each with its own set of T cells.

3) Notice now that there are **four** distinct antigens presented to the immune system P1, P2, P3 and P4.

4) Notice also that because all epitopes are **squares** there is some cross-reactivity denoted by a dashed line. **We have only shown X1-P2** although **all** possible X-P interactions are present.

5) How does this system evolve?

6) Without cross-reactivity we merely have four basic models, each with distinct antigens!

7) Adding cross-reactivity couples together together the four basic models.

§23. Cross-Reactive Evolution

1) In this solution variants 1 and 2 are not recognised by the immune system because they are not sufficiently immunogenic.

2) The immune response shows switching immune peaks denoted X3 and X4.

3) If all variants were not recognised we would expect a **relative abundance of 4** because there are 4 distinct antigen variants.

4) X3 and X4 T cells will reduce this to 2 (P1 + P2) plus some small amounts P3 + P4.

5) Cross reactivity reduces P1 and P2 still further.

6) **The generation of a cross-reactive response is an effective strategy enabling some recognition of even weakly immunogenic epitopes.**

So far the immune system has had it all its own way, immunodominance and cross-reactivity both serve to reduce parasite abundance. However the parasite can fight back:

§24. Parasite Antigen with Two Epitopes, One with Genetic Variation

1) We can now combine the ideas of multiple epitopes and genetic variation.

2) In this model we begin with an antigen P11 with two epitopes, **Red** and **Green.**

3) Some time later the **Red** epitope generates a new **Orange** variant. This introduces a second distinct antigen P12.

4) What can we say about the evolution?

   a. We know from Immunodominance, that the most immunogenic epitope will be recognised.

   b. If the **Green** epitope is immunodominant then a new **Red** variant **will not** be recognised (because the old one was not).

More...

c. **However** if the **Red** epitope is immunodominant then both the **Red** and the **Orange** epitopes **will** be recognised introducing competition.

d. This competition **may** give the **Green** epitope an advantage causing a **shift** in immunodominance to the **Green** epitope.

§25. Shifting Immunodominance (Parasite Abundance)

1) In this example the **Red** epitope is immunodominant.

2) At time t=5 the **Red** epitope generates an **Orange** mutation which is **more** immunogenic than the **Green** epitope. single mutant

3) Increased parasite abundance after infection with both P11 and P12 variants.

§26. Shifting Immunodominance (Immune Response)

1) Looking at the immune response shows this shift very clearly.

2) **Red** T cells are immunodominant until the **Red** epitope mutates giving the **Green** T cells a competitive advantage.

§27. Shifting Immunodominance

1) The immune system attempts to minimise the total parasite burden by selecting the **most immunogenic** epitope.

2) However **immunogenicity** is a combination of **two** factors:

   a. Proliferation,

   b. Variability.

3) Highly immunogenic epitopes can avoid recognition by genetic variation

4) Conserved weakly immunogenic epitopes can be recognised

§28. Three Epitopes

1) As a finale we can add still **more** epitopes and **more** variation.

2) In this example we have **three** epitopes **each** with variation.

3) In this example, the highly variable **Green** epitope is the most immunogenic.

4) There are now a total of **2 x 4 x 2 = 16** distinct antigens present.

§29. Masking of Immunogenic Epitopes

1) What we find when we solve the model is that, although the green epitope is most immunogenic, its variability can prevent its recognition by the immune system.

2) We call this process **masking**.

3) In fact the lowest parasite abundance is achieved by recognising the **yellow** epitope most strongly.

§30. Masking of Immunogenic Epitopes (Simulations)

1) Looking at the immune response shows that **both red** and **yellow** T cells have recognised antigen, whilst the **green** epitope has been completely masked

§31. Conclusions

1) Genetic variation makes the immune system much more complex.

2) Our last example showed both:

   a. **Masking** of an immunogenic epitope,

   b. **Coexistence** of different sets of T cells (**red** and **yellow**).

3) **The immune system can use competition as a means to minimise parasite abundance**

4) The **model** shows a clear parasite survival strategy:

   Generate variability at immunogenic sites on antigens of key importance to survival and transmission.

5) Immunodominance has implications for the choice of candidate antigens.

§32. Further Work

1) Look at selection strategies for epitope selection for candidate vaccines.

2) Introduce population genetics framework into the model to enable epidemiological model.

3) In the laboratory Dr. Guy Barker is beginning to look at genetic variation in helminth antigens as a means of confirming predictions:

   a. Look for strongly recognised immune responses at conserved epitopes.

   b. Identification of highly immunogenic but variable epitopes which do not elicit immunodominant responses.

Immunodominance, Competition and Evolution in Immunological Responses to Helminth Infections

Daren Austin

Wellcome Centre for Epidemiology of Infectious Diseases, Oxford University

24 October 1995

Helminth Infections

- 70% of the population live in developing world where helminths are one of the commonest infections
- 1/3 harbour intestinal helminths such as *Ascaris lumbricoides* and *Trichuris trichiura*
- 200m infected world-wide with Schistosomiasis

Schistosomiasis

- Infection via contact with fresh water containing cercariae released from intermediate snail hosts
- Transformation to schistosomulum
- Worms migrate to intestine or bladder
- Adult worms survive within the host for 3-8 years, despite evidence of immune recognition

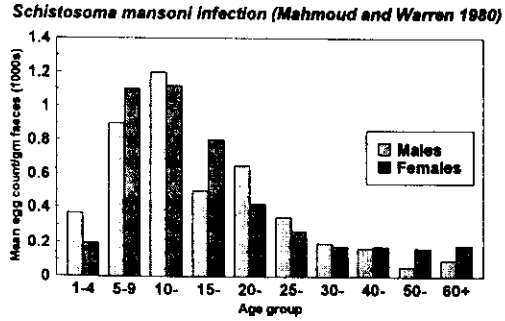
Vaccine Development

- Effective in reducing transmission
- Partial immunity in rodent models
- Some antigens identified and major B and T cell epitopes mapped
- MAP of Triose-Phosphate Isomerase shows recognition in mice strains

Field Studies

- Intensity rises during first two decades then declines in adults who show lower levels of reinfection
- Differences not accountable by exposure effects alone suggesting acquired immunity
- Blocking IgG and IgM antibodies in children prevent immunity
- Progressive increase in IgE levels with age

Schistosoma mansoni infection (Mahmoud and Warren 1980)



Immune Responses

- Humoral and cellular components most evident during infection
- Two subsets of differentiated T helper cells which are cross inhibitory
  - Th1 cytokines are IFN-γ and TNF-β
  - Th2 cytokines are IL-4, IL-5 and IL-10
- Imbalanced Th1/Th2 ratio characteristic

Modelling the Immune System

Population dynamics within the host

Modelling Aims

- Aim to capture the essence of the parasite-host immunological defences
  - Antigens are secreted, excreted and surface molecules
  - T cells proliferate in response to parasite antigen
  - Each antigen has a number of epitopes present
  - Epitopes are subject to some genetic variation

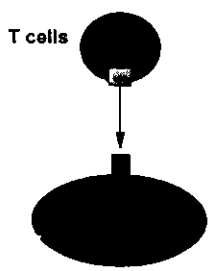
Model Assumptions

- Repeated exposure to infection at a constant rate
- No discrimination between humoral and cellular components
- No differentiation between Th1 and Th2 subsets
- T cells undergo clonal expansion on contact with antigen (or via presentation of antigen by APCs)

Model Assumptions 2

- Abundance of APCs is related to the parasite abundance
- Abundance of T cells is proportional to the effector arm of the immune system
- Immune system reduces parasite life expectancy not parasite establishment

Basic Model of a Cellular Response to Helminth Antigens



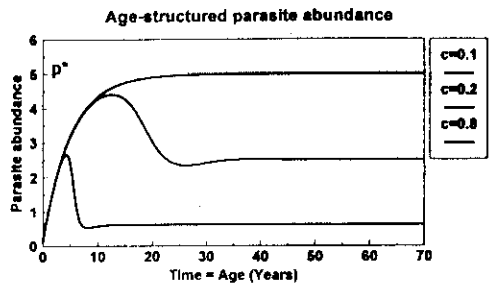
Basic Model

$$dp(t)/dt = \Lambda - \mu\, p(t) - h\, x(t)\, p(t)$$

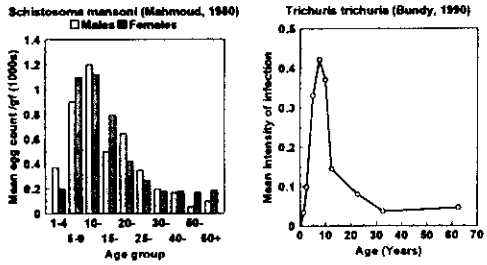
$$dx(t)/dt = c\, p(t)\, x(t) - b\, x(t)$$

- Λ = parasite immigration rate (= r p(t) for viral infections)
- μ = parasite death rate
- h = per-cell mortality rate
- c = per capita T cell proliferation rate (immunogenicity)
- b = T cell death rate

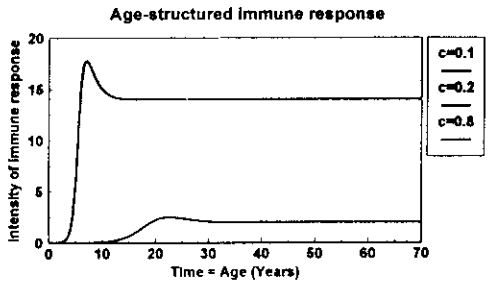
Numerical Evolution of the Basic Model Parasite Abundance



Age-related Intensity Profiles



Numerical Evolution of the Basic Model Immune Response



Summary of Basic Model

- In the absence of immune recognition the parasite abundance saturates to

$$\boxed{p(t) = p^* = \Lambda/\mu}$$

- Immunoactivation is only possible if epitope is sufficiently immunogenic

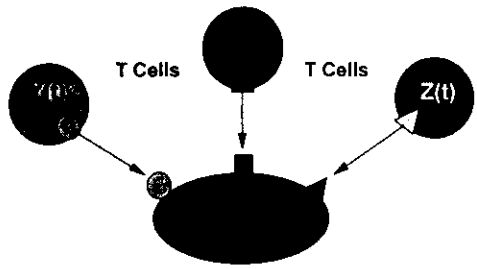
$$\boxed{c > b/p^*}$$

- Age of peak parasite intensity depends on c and initial T cell levels

More Realistic Models

Multiple epitopes and immunodominance
Genetic mutation of epitopes
Evolutionary strategies

Parasite Antigens with Multiple Epitopes



Equations!

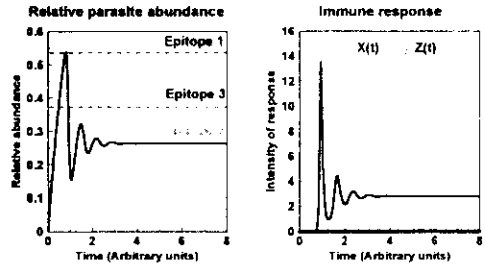
$$dP(t)/dt = 1 - P(t)\,(1 + X(t) + Y(t) + Z(t))$$

$$dX(t)/dt = \alpha\, X(t)\,(\beta 1\, P(t) - 1)$$

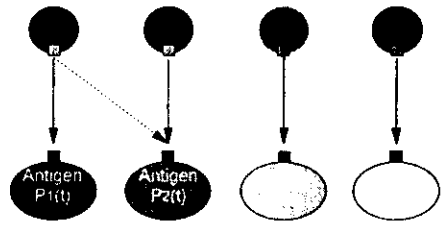
$$dY(t)/dt = \alpha\, Y(t)\,(\beta 2\, P(t) - 1)$$

$$dZ(t)/dt = \alpha\, Z(t)\,(\beta 3\, P(t) - 1)$$

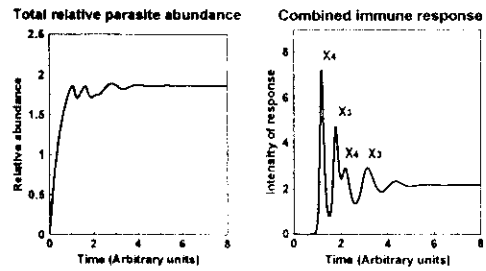
Immunodominance of One Epitope



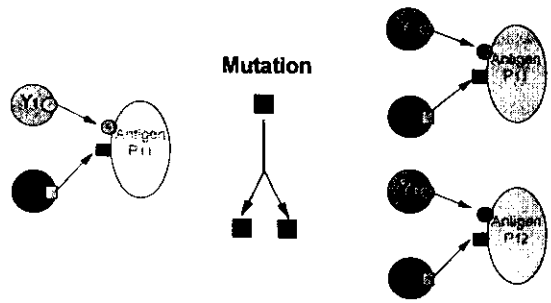
Genetic Variation at a Single Cross-Reactive Epitope



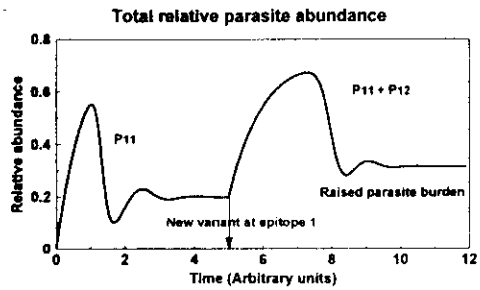
Cross-Reactive Evolution



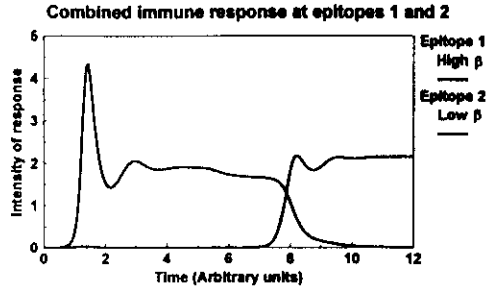
Parasite Antigen with Two Epitopes, One with Genetic Variation



Shifting Immunodominance
Parasite Abundance



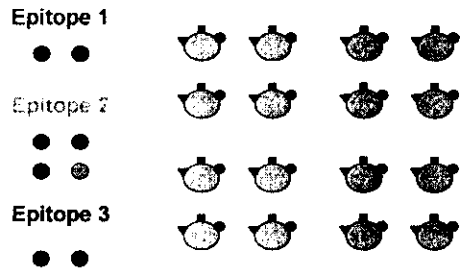
Shifting Immunodominance
Immune Response



Shifting Immunodominance

- **Immune system attempts to minimise the total worm burden via the most immunogenic epitope**
- **Immunogenicity is a combination of T cell proliferation *and* variability**
  - **Highly immunogenic epitopes can avoid recognition by genetic variation**
  - **Conserved weakly immunogenic epitopes may then be recognised**

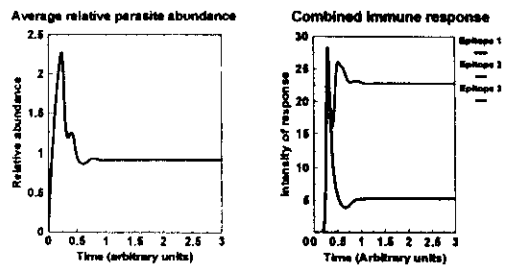
Three Epitopes



Masking of Immunogenic Epitope

- Epitope 1 has 2 variants
- Epitope 2 has 4 variants
  - Most immunogenic variant present at this site
  - Largest genetic variation causes masking of response
- Epitope 3 has 2 variants
  - Immunodominant epitope minimises parasite abundance

Masking of Immunogenic Epitope



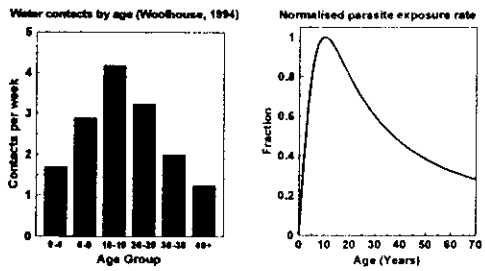
Conclusions

- Genetic variation makes the immune system much more complex
- The immune system is trying to minimise parasite abundance
- A clear parasite survival strategy is evident from the model
- Immunodominace has implications for the choice of antigens for candidate vaccines

Further Work

- Look at strategies for epitope selection for candidate vaccines
- Introduce a population genetics framework to enable epidemiological modelling
- Investigate genetic variation in helminth antigens as a means of confirming predictions

Modelling Parasite Exposure $\Lambda(t)$



Modelling Parasite Exposure $\Lambda(t)$ Parasite Abundance