



NC3Rs/POEMS Network Maths Study Group - Applying mathematics to 3Rs problems

Modelling the regulation of immunoglobulin class switching to IgE and IgG in human B cells to reduce animal use

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Background to the Problem

Upon initiation of an immune response in the germinal centres of the lymph nodes, B cells can switch the isotype of the immunoglobulin they produce from IgM to one of the more "specialised" isotypes (IgG, IgE or IgA). This process, termed class switch recombination, requires a carefully choreographed series of events to take place including: gene transcription, DNA breakage and recombination, cell division and the regulation of apoptosis. Immunoglobulin class switch recombination to IgE can occur either directly from IgM or via IgG as an intermediate. We wish to investigate the kinetics of these reactions to understand which the rate limiting steps are and why production of IgE occurs less frequently than IgG. This will help us to better understand and target the mechanisms controlling immunoglobulin class switch in response to genuine and innocuous antigens.

Details of the problem

The production of specific immunoglobulin isotypes, tailored to respond to specific challenges, is central to the success of the vertebrate adaptive immune system and must be tightly regulated to maintain health. For example, the production of IgE is required to clear helminth infections, yet results in allergy when B cells recognising "innocuous" antigens switch to produce IgE in the nose (hay-fever), lung (asthma) or skin (eczema). Similarly, the dysregulated production of IgG lies at the heart of many immune conditions such as arthritis and autoimmune disease.

B cells switch to produce IgG, IgE or IgA through the recombination of the "variable" region of the Ig gene with one of the downstream "constant" region gene segments (1). Much is known about molecular pathways that carry out the recombination process (1) and the transcriptional networks that control them and the subsequent differentiation into Ig producing plasma cells (2). However, very little is understood regarding how class switching to one isotype or another is controlled to produce the required immune response. Class switch recombination takes place when B cells receive cytokine signals in the presence of T-cell help, initiating the transcription of the "germline" constant region genes and activation of the recombination pathway. It is known that cell division is required for the completion of class switching, and it has been proposed that division number may regulate the outcome of the process (3). However, the basis for this division-linked regulation is still poorly understood. Recently, much attention has been given to the mathematical modelling of the germinal centre reactions (4-6) and we feel that a similar investigation of the mechanisms directing class switching would be highly informative.

Ideas and data for informing possible mathematical models

- Immunoglobulin germline gene transcription;
- Transcription and nuclear import of the enzyme (AID) that initiates recombination;
- The recombination process itself (including following the recombination process on both alleles), investigating the kinetics of direct (IgM->IgE/IgG) and sequential (IgM->IgG->IgE) switching and proportion of cells that finally produce each isotype;
- Cell division (the rate and number of cell division as well as the proportion of cells in each phase of the cell cycle) and apoptosis.
- In addition a microarray analysis of gene expression has been undertaken to investigate the transcriptional changes underlying these events.

We now wish to investigate whether mathematical modelling can help establish which of these events are rate limiting and whether the kinetics of switching differ for IgG vs. IgE and direct vs. sequential switching.

Questions you would like to see answered

We would specifically like to gain information on:

1. Whether the final outcome of switching (the proportion of IgE vs. IgG producing cells) occurs due to differences in the kinetics of recombination to IgE vs. IgG or to inherent differences in the recombination frequency to IgE vs. IgG.
2. Determine the effect of cell division and apoptosis on directing the outcome of class switching.
3. Determine whether differences exist within the kinetics of direct (IgM-IgE) vs. sequential switching (IgM-IgG-IgE).

We would also like to use this process to identify possible future experimental design and data needs to address these questions specifically within a mathematical model, rather than in vivo.

The potential impact on animal use

To date much research in this area, particularly with respect to the regulation of isotype "choice" by the B cells has been carried out in mice, utilising in vitro stimulation of primary murine B cells (isolated post-mortem from the spleen and bone marrow) and mouse models of asthma and allergic disease. These studies use large numbers of animals: Typically 3-10 mice per in vitro experiment and up to 50 for a full in vivo disease model study (particularly if structural lung changes are investigated). In addition, these studies are associated with animal welfare concerns; asthma disease models require repeated dosing of inhaled allergen over the course of several weeks and long term kinetic investigations of circulating and lung lavage cell numbers over the course of months in mice with impaired life expectancy. However, we (and others) have shown that class switching appears to be regulated differently in mice vs. man and that as such mouse models of disease have not been predictive, particularly regarding asthma. We wish to extend the utility our human ex vivo culture system and demonstrate its potential to investigate complex regulatory networks. If the model development was successful we would be keen to explore its wider application in the immunobiology community to encourage uptake of human ex vivo tissue models and reduce reliance on animals.

Relevance to medicine and healthcare

The inappropriate or dysregulated production of different immunoglobulin isotypes underpins a number of human diseases that significantly affect patient morbidity and have serious economic impacts, including; asthma and allergy (IgE) and arthritis and autoimmune disease (IgG). However, to date very little is known about how class switching to particular immunoglobulin isotypes is regulated. A detailed understanding of these events (in human) will not only significantly contribute to a poorly understood area of immunobiology but could also highlight new therapeutic targets for the treatment of a diverse range of diseases.

References

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