Research Protocol

A comprehensive, long-term integrated programme to study the aetiology and immunopathology of childhood infectious, inflammatory and allergic disease, using the large patient base of children attending St Mary's Hospital, London and other collaborating sites

Chief Investigator: Professor Michael Levin

BACKGROUND – WHY IS THE RESEARCH NEEDED?

When children present to hospital unwell, we often find ourselves unable to answer basic questions about their illness. For instance, in the case of a febrile child (a child with fever), we are often unsure if the child has an infectious or non-infectious problem. In those children with infections, we are often unable to say whether the infection is bacterial, which might justify antibiotic use, or viral – let alone identify the infectious organism.

The key to accurate diagnosis – and therefore to appropriately targeted treatment – may come from a fuller understanding of two broad but connected questions.

Firstly: how does the body respond to an infection, or to a disease process? Can we perhaps make a diagnosis more accurately if we look at what genes and proteins are being used in that patient to fight the infection? For instance, could we distinguish between different viral and bacterial infections in a child by looking at which genes are 'switched on' in that infection?

Secondly: what are the genetic factors which determine how a child responds to an infection? For instance, why does one child develop life-threatening meningitis, but another child show no symptoms, even though both children started off with the same organism infecting their throat? Are there non-genetic factors related to the child's immune system which could contribute, for example nutritional status, or their recent exposure to other infections?

Scientific Justification for the research

There are exciting prospects that the first of the above questions can be tackled by using gene expression technology (including gene arrays) to examine what genes are expressed in cells from blood, when a child fights an infection, and an important landmark paper from 2006 established that analysis of only 35 genes could discriminate accurately between different types of infection (Ramilo et al., 2007). However, there has in fact been relatively little work to date using gene expression profiling to discriminate between infections in children. This is an exciting area to explore further, and Prof. Levin's group has a proven track record of using gene arrays to explore the importance of variation in gene expression and its relation to illness (Griffiths et al., 2005; Kampmann et al., 2005; Pathan et al., 2004).

In considering the second question, what are the host determinants of the response to infection, Prof. Levin's group have demonstrated that translational research based on patients seen at St Mary's hospital can lead to significant advances in our understanding of how children fight infection, through the detailed work-up of individual patients who have had severe infection (see for instance (Kampmann et al., 2005; Newport et al., 1996)). The proposed project would build on this work.

Inflammation

In considering the contribution of inherited genetic variation to disease susceptibility, Prof. Levin's group has a strong interest in inflammatory illness in children, particularly Kawasaki disease. Recruitment of children with such conditions would contribute to on-going research programmes in this area (Burgner et al., 2009). A re-appraisal of how genetic data can be analysed has led to the devising of a new approach which strengthens the yields of genome-

wide association studies (Burgner et al., 2009; Eleftherohorinou et al., Submitted). In time, we would be keen to exploit the expertise in this area to new cohorts of patients.

Allergy

Allergic diseases are the commonest chronic diseases of childhood in the UK, and continue to increase in prevalence worldwide at an alarming rate. Its ontogeny remains poorly understood. Prof. John Warner's interest in the field of developmental immunology and allergy has led to many contributions in the field of the early life origins of allergic disease (Jones et al., 2002). There is a busy tertiary paediatric allergy service at St Mary's Hospital, with a very active research base. There is considerable interest in the interactions between infectious, allergic and inflammatory diseases in early childhood.

Current major research interests in the department include the pathogenesis and characterisation of food hypersensitivity, the treatment of asthma and the primary prevention of allergic disease (McCann et al., 2007). Routine serum samples are taken from children attending the allergy clinics, and we would like to take additional samples for the characterisation of allergic immune responses and genetic and epigenetic correlates of allergic disease.

Projects with the Centre for Respiratory Infection (CRI)

This project is supported by the Centre for Respiratory Infection, based at Imperial College (including at St Mary's site). This world-class association of clinicians and scientists includes several groups working on the immunopathology of respiratory infection and allergy, and the project will capitalise on the expertise of these groups, both for enhanced diagnostic work on patient samples, and also for scientific lines of enquiry. The location of St Mary's Hospital means that it will greatly benefit from input from CRI teams, not least because many patients enrolled in the study will have respiratory infections and allergic airways disease. Below are some examples of how patients recruited will contribute to studies at the CRI.

Some babies with bronchiolitis caused by RSV (a common viral cause in babies) have a more severe course of infection than others. One possibility for this is that babies with more severe RSV illness in fact have multi-pathogen infections (see (Brunstein et al., 2008)), and collaboration with CRI labs will allow an intensive search for additional pathogens together as well as a parallel approach in which the contribution of the antiviral immune response is considered (Openshaw, 2005).

Collaborative work with groups in the CRI will also allow us to monitor influenza strains in circulation in the paediatric population attending St Mary's hospital. Any influenza strains identified in the course of routine virology investigations will be further analysed by Dr Wendy Barclay's group. This surveillance will contribute to maintaining a state of preparedness for an outbreak of pandemic influenza

The CRI has a strong research base in childhood tuberculosis. Recruitment of patients will contribute to the on-going studies in the department which aim to identify serum biomarkers or gene expression profiles that can distinguish patients with TB infection or disease, with a view to improving diagnosis (Newton et al., 2008).

In children with severe bacterial respiratory infection, we are interested in the extent to which lung inflammation and injury is influenced by recent viral infection, as seen in bacterial pneumonias following influenza infection. There is evidence from mice to suggest that after viral infection, there is prolonged down-regulation in the response of the innate immune response to subsequent infections, with the consequence that these infections cause more damage than they would normally (Didierlaurent et al., 2008). Local changes in immune responsiveness may also be important determinants to the outcome of respiratory infection with Pneumocystis jirovecii (the cause of pneumocystis pneumonia) in HIV-infected children (Thomas and Limper, 2007). Using BAL or bronchial brushing samples, we would like to examine the immune milieu of children with severe pneumonia caused by different pathogens, using multiplex cytokine analysis and lymphocyte functional characterisation. We will correlate data from bronchial samples with investigations on peripheral blood cells, in

order to determine whether the local immunological changes in the lung are mirrored in peripheral blood.

Principal Objective

We will study a large cohort of children with a broad range of infectious, inflammatory and allergic diseases. We would like to know if we can improve the diagnosis rate in this group of patients, and we will use the latest microbiological approaches to increase our chance of identifying any infection.

We would like to further our understanding of how children respond to infectious, inflammatory and allergic disease at a genetic, proteomic and cellular level. We will measure these responses using a variety of immunological, molecular biology and proteomic techniques, and correlate the results with the clinical findings. We would therefore hope to develop an increased understanding of disease aetiology. In summary, the principle questions are as follows:

1. What are the bacterial and viral causes of acute illness in children presenting to a UK general hospital, tertiary paediatric infectious disease unit and paediatric intensive care unit?

2. What immunopathogenic mechanisms are involved in severe infection caused by specific bacterial and viral pathogens, and in inflammatory and allergic conditions?

Secondary Objectives

Secondary objectives are considered as sub-headings of the primary objectives

1. What are the bacterial and viral causes of acute illness in children presenting to a UK general hospital, tertiary paediatric infectious disease unit and paediatric intensive care unit?

• Can we define what proportion of infections are viral and which are bacterial?
• What is the relationship between viral respiratory infection and bacterial superinfection?
• What role do infections play in precipitating or extenuating asthma and allergic lung diseases?
• To what extent does dual infection contribute to increased morbidity?
• Can the microbiological diagnosis of acute infection in children be improved by application of new diagnostic methods including PCR assays, gene expression profiling, multi-parameter flow cytometry and proteomics?

2. What immunopathogenic mechanisms are involved in severe infection caused by specific bacterial and viral pathogens, and in inflammatory and allergic conditions?

• To what extent are host inflammatory responses vs. bacterial or viral load important in host injury in infection?
• What insights do proteomic methods (such as mass spectrometry) and gene expression analysis give into immunopathology of infection?
• To what extent does heritable genetic variability determine the host response in infection, and does it underlie a predisposition to severe disease?
• Does heritable genetic variability underlie a predisposition to inflammatory and allergic conditions?
• To what extent do the immunopathogenic mechanisms identified in animal studies apply in human disease?
• To what extent is immune system function governed by non-heritable factors, such as dietary and nutritional status?

In addition to the above, we anticipate that the development of robust and systematic methods to recruit considerable numbers of children into this research programme will create

a useful pathway that would be applicable to patients presenting to St Mary's Hospital with emerging infections, such as PVL staphylococcal disease or emerging viral infections.

Inclusion Criteria

St Mary's Hospital

- Children presenting via any means to St Mary's Hospital; this would include the A&E department, the general and infectious disease wards and the paediatric intensive care unit.
- Children needing blood tests for any clinical reason (ie we will not take blood samples from children who would not otherwise be having them taken).
- Children who, in the clinical judgement of the doctor assessing them, have presented because of a condition consistent with an infectious, inflammatory or allergic process.
- We will recruit well children into this study to act as controls. These will be children who are in any case having blood tests, for instance children undergoing elective surgery

Collaborating sites

- Children needing blood tests for any clinical reason (ie we will not take blood samples from children who would not otherwise be having them taken).
- Children presenting at collaborating sites with a proven or suspected respiratory infection **or** proven or suspected infection with a pathogen associated with respiratory disease without respiratory manifestations.

Exclusion criteria

- Children must be aged 16 years or younger.
- Children re-presenting with the same condition would not be re-enrolled, but they could be re-enrolled if they have a new condition.
- If there is a concern that the requirements of the study are not fully appreciated by the parent/guardian or child due to for example a lack of comprehension of information given in English then they will be excluded.

Sample Size

We would hope to enroll 1000 patients over a 3 year period, with a range of diagnoses. The methods of gene expression and proteomic biomarker detection require groups of 30-50 patients within each diagnostic category (for instance viral infection vs. bacterial infection), in order to have adequate power for analysis.

Confirmatory studies on twice this number are required for validation.

However, even rare presentations of disease would be of interest as individual or small groups are amenable to analysis at a molecular level.

METHOD

Enroling patients.
When the research project is going to start, we will educate the paediatric team working at St Mary's Hospital or a collaborating site, including dedicated research nurses, about the project. We will ensure that the scientific basis for the project, the entry criteria and the consenting process are clearly understood.

If members of the paediatric team believe that a patient they are assessing qualifies to enter the study, they will be encouraged to enrol them either directly themselves, or by asking a member of the clinical research team to come and consent the child and take bloods (for

instance a research nurse or clinician scientist). The project will be explained using an age-appropriate participant information sheet.

The consenting procedure
This will involve the following. We will explain to children and their carers that there is a clinical need for a blood test, and that we would like to take a small extra volume of blood at the same time for a research project. In addition, if other samples are being collected for clinical reasons, such as urine or nasopharyngeal aspirates, if the sample volume is sufficient we will seek consent to take an aliquot for research purposes. We may seek consent to collect additional non-invasive samples for research purposes, such as urine samples or throat swabs, in some cases where there is not a clinical need to collect these.

We will recruit well children into this study to act as controls. These will be children who are in any case having blood tests, for instance children undergoing elective surgery. This will be important in order to give us a baseline comparator. Informed consent will be sought in all instances.

Blood sample collection
Sample collection will be performed either by the clinical team or research nurses, if consent is given. The normal clinical bloods will be taken along with additional research samples, with the following volumes:

Child above 5 years
Serum – 4ml, EDTA – 4ml, PAX – 2.5ml

Children below 5 years
Serum – 1ml, EDTA – 1ml, PAX – 2ml
(We recognise that these volumes will not always be obtainable in smaller children.)

Serum samples will be spun and frozen for proteomic work, such as biomarker characterisation.
EDTA samples will be frozen pending DNA extraction for genomic analysis.
RNA (PAX tubes) will be frozen pending RNA extraction for gene expression analysis.

We will also collect 4ml blood samples for extraction of cells (for instance neutrophils or lymphocytes) or plasma when there is the laboratory capability for immediate processing.

Other samples
Other samples being taken for clinical reason, such as CSF, will be aliquoted for research purposes if there is sufficient. Simple non-invasive tests such as urine collection, throat swab or collection of nasal mucous will be collected in the absence of obvious clinical need if there is fully informed consent.

Where children in the intensive care unit are having bronchoalveolar lavage (BAL) or bronchial brushings for a clinical indication, we will aim to aliquot the sample for research as described.

Processing samples.
Before samples are processed in the laboratory, they will be labelled with a study number, and personal details will be removed. We would not ordinarily expect any further participation in the study after the first time bloods have been taken from the patient.

During their in-patient stay, we will follow the clinical progress of each child in order to document clinical outcome. We may seek consent for additional samples during the patient stay.

Surplus samples.

In the event that clinical samples are taken from a child before there has been an opportunity to enrol them into the study, for instance overnight, we may approach the family to enrol the child. If consent is given, we will endeavour to use any surplus blood or microbiological samples from the clinical diagnostic laboratories.

In this study, it is not necessary that the range of children entering into the study be representative of the clinical range of children with infectious, inflammatory or allergic conditions. Our patient base is in any case distorted by the fact that a disproportionate number of children will be so ill as to need intensive care admission, and the remainder will have been brought to hospital and be ill enough to warrant blood tests. Therefore, we do not expect researcher bias to be a problem. We will keep a clinical dataset for each patient entered into the study so that test results are interpreted in the light of the clinical presentation.

Sample Analysis

1) We will use state-of-the-art diagnostic techniques, which are not ordinarily available for routine use in hospital, to look for infections in blood and in other samples if they are taken, such as cerebro-spinal fluid (CSF). These tests include polymerase chain reaction (PCR).

2) We will look at the range of proteins that are present in the children's blood - infections or diseases may be defined by the 'signature' of proteins in the blood.

3) We will look at which genes are being used by the white cells in the blood to fight infection in order to understand how the body is responding. We may also be able to diagnose an illness by looking at which genes are being used by the white cells.

4) We will look for differences in the genes (part of the DNA) between patients which might explain why two patients behave differently - for instance, why does one child get a sore throat but another child become seriously ill with the same infection?

5) Under a variety of laboratory conditions, we will look at how cells from the patient are functioning from an immunological point of view; for instance we will identify which cytokines (immune messenger molecules) they are making.

Statistics and Data Analysis

Sample size: Among patients presenting with symptoms of infection there will be groups of patients with common disorders such as RSV bronchiolitis and bacterial pneumonia, however much smaller numbers of patients will present with rare but important infections such as PVL staphlylococcal infection, toxic shock or Kawasaki disease.

Our enrolment procedure will enable all patients to be included in the study, and as a result, there will be large groups of patients with common disorders and small numbers of patients with rare disorders.

In this regard, a formal power calculation is not possible; however, to obtain disease-specific genetic signatures a minimum of 30-50 patients in each group are required, with validation in a larger number.

Methods of analysis: Analysis of complex immunological data, including gene expression and proteomic analysis, will be undertaken in collaboration with the department of epidemiology and biostatistics at Imperial College. Prof Levin has on-going collaboration with Prof David Balding, who is a leader in application of statistical methods to large biological datasets.

Data Retention

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

REGULATORY ISSUES

Ethics Approval

The Chief Investigator has obtained approval from the St Mary's Research Ethics Committee. The study will be submitted for Site Specific Assessment (SSA) at St Mary's Hospital. The Chief Investigator will require a copy of the SSA approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Consent

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained for all participants agreeing to take part in the study. The right of the participant and their parent/guardian to refuse to participate without giving reasons will be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and will follow the Data Protection Act.

Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Sponsor

Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

Funding

The research is funded by the European Commission

Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and compliance with the NHS Research Governance Framework for Health and Social Care (2nd edition).

Study Management

The day-to-day management of the study will be co-ordinated through the Chief Investigator.

Publication Policy

All publications and presentations relating to the study will be authorised by the Chief Investigator. If there are named authors, these will include at least the trial's Chief Investigator and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the main research

team will be according to the individuals involved in the project but must acknowledge the contribution of any of the main study researchers involved.

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