IgG SUBCLASS CONCENTRATIONS IN CHILDREN IN HEALTH AND DISEASE

A thesis submitted by

LORRAINE JOYCE BEARD, M.B.,Ch.B., F.R.A.C.P.

to the University of Adelaide,
South Australia

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Department of Paediatrics,
University of Adelaide,
South Australia

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TABLE OF CONTENTS

THESIS SUMMARY

Athough the existence of four isotypes of IgG, known as IgG subclasses, has been recognised for over 20 years, there is still a great deal of confusion about both the definition and the significance of abnormal IgG subclass concentrations. This is partly because accurate measurement of the IgG subclasses is difficult and results have often been unreliable. Accurate normal ranges for patients of different ages have, therefore, been difficult to establish. Studies of patients with subclass abnormalities have been largely anecdotal. It has, however, been suggested that IgG subclass deficiencies may prove to be the most common form of primary immunodeficiency.

In many children who suffer recurrent infections, standard immune function studies including measurements of IgA, IgG and IgM, neutrophil and lymphocyte function studies and serum complement screening fails to detect a particular immunological problem. Some investigators have suggested that an IgG subclass deficiency may exist in a considerable proportion of these patients and may be associated with impaired production of antibodies to a variety of antigens. Other studies suggest that IgG subclass deficiency may not be an important factor. The chief aim of this project was to help to clarify some of this confusion by developing a reliable enzyme-linked immunosorbent assay (ELISA) technique and using this to:-

a) establish percentile ranges for IgG subclasses in healthy Australian children.

b) quantitate serum IgG subclasses in groups of patients with differing types and degrees of infection proneness.

c) quantitate serially serum IgG subclasses in children with immunodeficiency states who are receiving regular immunoglobulin infusions.

These studies have increased our understanding of the relationship between IgG subclass deficiencies and infection proneness in children, and have resulted in suggestions for directions for further work in this area.

The ELISA technique was developed using monoclonal antibodies to each of the IgG subclasses.

Serum samples from healthy Australian children were used to determine age-related percentile ranges for IgG subclasses. Previous studies have generally used less sensitive assay techniques and have not been able to define the lower limit of normal for IgG4 which is found in relatively low concentrations in many normal sera. We were able to quantitate IgG4 in all our healthy subjects..

The relationship between IgA deficiency and IgG subclass deficiency was studied. In a preliminary study we found that while infection-prone children with IgA deficiencies were more likely to have IgG subclass deficiencies than were non-IgA deficient children, those with profound IgA deficiencies were less likely to have IgG subclass deficiencies than those with less profound IgA deficiencies. In this preliminary study, IgG subclass quantitation was done by electroimmunoassay and we were unable to determine the incidence of IgG4 deficiency because of limitations in the sensitivity of the assay. In a second study, we found IgG4 deficiency to be the most common IgG subclass deficiency associated with IgA deficiency.

In two other groups of patients, low IgG4 concentrations were not uncommon. Of fifteen patients with bronchiectasis, 27% were IgG4-deficient and a group of children with invasive *Haemophilus influenzae* type b infections had low, or relatively low, IgG4 concentrations.

However, IgG subclass deficiencies were not common in groups of children with osteomyelitis or septic arthritis or giardiasis. IgG subclass deficiencies, and particularly those of IgG4, appeared more often amongst patients with proneness to infections of the respiratory tract, or infections with pathogens gaining entry via the respiratory tract, than amongst the patients with other types of infections.

To contribute to the understanding of the control of IgG subclass production we quantitated IgG subclasses in children with a variety of immunological disorders where control mechanisms are dysfunctional.. We also studied the effect of the spleen on IgG subclass concentrations by quantitating IgG subclasses in two groups of splenectomized patients and in a small group of children with portal hypertension.

Finally, we studied the IgG subclass composition of several intravenous immunoglobulin preparations and then investigated the effect of immunoglobulin replacement therapy on IgG subclass concentrations in patients with either hypogammaglobulinaemia or IgG subclass deficiency. While most

infection-prone patients with either hypogammaglobulinaemia or IgG subclass deficiency without generalised hypogammaglobulinaemia showed marked clinical improvement with regular immunoglobulin replacement therapy, the degree of improvement did not always parallel the improvement in the IgG subclass concentrations.

Overall, the relative frequency of IgG subclass deficiencies in the patients studied was: IgG4 44%, IgG2 22%, IgG1 18% and IgG3 15%. The frequency order was the reverse of the order of the IgG subclass heavy chain gene sequence, suggesting that the greater the amount of downstream isotype switching required, the more likely is a deficiency. The combination of isotype deficiencies found most frequently was that of IgA and IgG4. While this suggests a defect in isotype switching at the genetic level, the fact that IgG2 deficiency occurred much less often indicates that more than only an arresting of isotype switching is involved. Even in patients deficient in one or more IgG subclass isotypes, the deficient isotype was always detectable, indicating that the defect in production was probably regulatory rather than structural.

While IgG subclass deficiencies are associated with proneness to some types of infections in children, further studies are necessary to clarify the relationship and to develop optimal ways of treating infection-prone IgG subclass-deficient patients. Potentially useful areas of future study would include:-

(i) relationships between IgG subclass deficiencies and functional antibody deficiencies.

(ii) the importance of IgG subclasses in mucosal secretions.

(iii) mechanisms of controlling IgG subclass production.