IMMUNOGLOBULIN PROFILES IN HEALTHY AND IN HUMAN IMMUNODEFICIENCY VIRUS type 1 SEROPOSITIVE INDIVIDUALS IN DAR ES SALAAM, TANZANIA.

ELIGIUS FRANCIS LYAMUYA, M.D(Dar)

MEDICAL LIBRARY · DAR ES SALAAM

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF MEDICINE (MICROBIOLOGY) IN THE UNIVERSITY OF DAR ES SALAAM

1991

IMMUNOGLOBULIN PROFILES IN HEALTHY AND IN HUMAN IMMUNODEFICIENCY VIRUS type 1 SEROPOSITIVE INDIVIDUALS IN DAR ES SALAAM, TANZANIA.

ELIGIUS FRANCIS LYAMUYA, M.D(Dar)

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE DEGREE OF MASTER OF MEDICINE (MICROBIOLOGY) IN THE UNIVERSITY OF DAR ES SALAAM

1991

SUPERVISORS

PROF.S.Y. MASELLE
University of Dar es Salaam

PROF.ROALD MATRE
University of Bergen

DECLARATION

I, ELIGIUS FRANCIS LYAMUYA, HEREBY DECLARE THAT THIS DISSERTATION IS THE PRODUCT OF MY OWN WORK, AND THAT TO THE BEST OF MY KNOWLEDGE, IT HAS NEVER BEEN SUBMITTED FOR A DIPLOMA OR DEGREE IN ANY OTHER UNIVERSITY.

Signed.....

Candidate

1991

Signed.....

Supervisor

S.Y.Maselle

Associate Professor, Microbiology/Immunology, Muhimbili Medical Centre, University of Dar es Salaam.

1991

COPYRIGHT (C) 1991

All rights reserved. No part of this dissertation may be replaced, stored in any retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without prior written permission of the author or the University of Dar es Salaam in that behalf.

DEDICATION

This dissertation is dedicated to my beloved mother, Augustina, who instilled in me the virtue of patience.

`The search for truth is in one way hard and in another way easy,
For it is evident that no one can master it fully, nor miss it wholly,
But each adds a little to our knowledge of nature,
And from all the facts assembled, there arises a certain grandeur'.

Aristotle.

TABLE OF CONTENTS

Page

List of contents.....v

List of tables.....viii

List of figures.....x

Acknowledgements.....xi

Summary.....xiii

1.0 Introduction and literature review.....1

1.1 Introduction.....1

1.2 Immunoglobulins.....2

1.2.1 Structure.....2

1.2.2 Production.....5

1.2.3 Electrophoretic pattern.....7

1.2.4 Functions.....9

1.2.5 Classes.....9

1.2.6 Serum levels.....13

1.3 Subclasses.....14

1.3.1 IgG subclasses.....15

1.3.1.1 Historical perspective.....15

1.3.1.2 Physicochemical properties.....15

1.3.1.3 Biological properties.....17

1.3.1.4 Distribution in normal individuals.....20

1.3.1.5 Subclass responses to various antigens.....24

1.3.1.6 Subclass responses in various disease states.....25

Page

1.3.1.7 IgG subclass deficiency.....26

1.3.1.8 Quantitative determination of IgG class and subclass levels.....27

1.4 HIV-1 infection and the immune system.....28

1.4.1 Historical perspective.....28

1.4.2 Agent.....29

1.4.2.1 Morphology.....29

1.4.2.2 Molecular organisation and antigenic structure...29

1.4.2.2.a The structural HIV-1 genes and their encoded proteins.....29

1.4.2.2.b HIV-1 regulatory proteins.....31

1.4.3 Pathogenesis of HIV-1 infection.....31

1.4.3.1 Mechanism of infection.....31

1.4.3.2 Immunological abnormalities.....32

1.4.3.2.a Defects in cellular immunity.....32

1.4.3.2.b Abnormalities of the humoral immune response.....35

1.4.4 The host immune response in HIV-1 infection.....37

1.4.4.1 Humoral response.....37

1.4.4.2 Cellular immune response.....39

1.4.5 Immunological tests for HIV-1 infection.....40

1.4.5.1 Antibody detection.....40

1.4.5.2 Antigen detection.....41

2.0 Rationale of the study.....42

Page

3.0 Objectives....44
3.1 Broad objective....44
3.2 Specific objectives....44
4.0 Materials and methods....45
4.1 Subjects....45
4.2 Serum samples....46
4.3 HIV-1 serology....46
4.4 Estimation of C-reactive protein....47
4.5 Quantitation of IgG, IgA, and IgM....47
4.6 Quantitation of IgD and IgE....47
4.7 IgG subclass assay....49
4.8 Assay of IgG subclass response to gp41 and p24....51
4.9 Statistical analysis....52
5.0 Results....53
5.1 C-reactive protein estimation in healthy individuals...53
5.2 Immunoglobulin levels in maternal and cord sera....53
5.3 Immunoglobulin levels in HIV-1 seronegative blood donors and in HIV-1 seropositive individuals....55
5.4 Anti-HIV-1 IgG subclass response to gp41 and p24....59
6.0 Discussion....60
7.0 Conclusion....73
8.0 Recommendations....75
9.0 References....76

LIST OF TABLES

Page

1. Physical properties of major human immunoglobulin classes....12

2. Biological properties of major human immunoglobulin classes..13

3. Human IgG subclasses: Physicochenical properties.....17

4. Human IgG subclasses: Biological properties.....20

5. Mean levels of immunoglobulins in maternal and cord sera.....53

6. Mean levels of IgG subclasses in maternal and cord sera.....54

7. Fetal/maternal ratios for total IgG and IgG subclasses at term.....55

8. Mean serum levels of immunoglobulin classes in HIV-1 seronegative controls and in HIV-1 seropositive subjects.....53

9. Mean serum levels of immunoglobulin classes in asymptomatic HIV-1 seropositive individuals and in AIDS patients.....56

10. Mean serum levels of IgG subclasses in HIV-1 seronegative blood donors and in HIV-1 seropositive subjects.....57

LIST OF FIGURES

Page

Fig. 1 The basic structure of an immunoglobulin molecule.....4

Fig. 2 Schematic representation of a densitometric tracing of serum proteins separated by electrophoresis.....8

Fig. 3 Development of IgG subclasses in children.....23

ACKNOWLEDGEMENT

First and foremost, I am greatly indebted to my supervisors: Prof.S.Y.Maselle (University of Dar es Salaam) initiated the study by introducing the study topic to me, constantly went through my work with dedication and gave me valuable constructive criticisms. Prof.R.Matre (University of Bergen, Norway) gave me excellent guidance throughout the different stages of the work. I particularly acknowledge his inspiring collaboration, his never-ending enthusiasm, and his constant encouragement and scientific critique. Prof.F.S.Mhalu and Prof.J.F.Shao are acknowledged for teaching me the various aspects of Microbilogy, and for their encouragement during the preparatory stage.

Prof.Per Oeding deserves special thanks for making my stay in Norway comfortable. His fatherly benevolence, and his kindness enabled me to withstand the inevitable depression of staying very far away from my family. I also thank Prof.Tor Hofstad and Prof.Gunnar Haukeness for their invaluable contribution in facilitating my work in one way or another. I acknowledge Prof.Rune Nilsen's enthusiastic encouragement during my stay in Bergen.

Much of the work demanded thorough laboratory tests. All technicians in the serology section and Broegelman Research Laboratory in Microbiology, (department of Microbiology and Immunology, The Gade Institute, University of Bergen) are deeply acknowledged for assisting me in many ways during my practical training in Immunology as well as during my dissertation work. Many thanks are due to the following technicians for their invaluable assistance: Inger Grimelund, for teaching me the technical aspects of ELISA test; Irene Ovidia

Berentsen, for making available to me all necessary reagents; Turid Tynning, for teaching me IgG subclass ELISA; Marianne Eidsheim, for her memorable assistance during the IgG subclass assays; Eli Hegrenaes, for assisting me with nephelometric assay; and Dagny Spissoy, for allowing me to work in her laboratory and placing all important facilities at my disposal. I would also like to thank Brit Nygaard and Wencke Davicknes for assisting me in securing most of the references quoted in this work.

I wish to thank the Norwegian Agency for International Development (NORAD) for the financial assistance which made this work possible. Their humanitarian gesture and their endeavour to foster scientific education in Tanzania is greatly appreciated.

I also wish to thank Dr.S.A.Dielly (Department of Microbiology and Immunology, Muhimbili Medical Centre) for providing me some of the sera from patients with AIDS. Finally, many thanks to my family for being understanding during my absence while I was in Norway. Not least important, their prayers did contribute a lot to my success in doing this work.

SUMMARY

Serum levels of IgG, IgA, IgM, IgD, IgE, and IgG subclasses were determined in 60 sera from pregnant mothers and their newborns, 50 sera from HIV-1 seronegative blood donors and 50 sera from HIV-1 seropositive individuals from Dar es Salaam. The mean levels of immunoglobulin isotypes in maternal sera were 19.9g/l, 2.79g/l, 3.08g/l, 68.33IU/ml, and 2689.33IU/ml for IgG, IgA, IgM, IgD, and IgE respectively. The mean IgG subclass levels in maternal sera were 11.99g/l, 3.74g/l, 2.38g/l, and 1.11g/l for IgG1, IgG2, IgG3, and IgG4 respectively. Placental transfer of all IgG subclasses was demonstrated. In the corresponding cord sera, the mean levels for total IgG and IgG subclasses were 16.82g/l, 12.77g/l, 0.86g/l, 2.11g/l, and 0.68g/l for total IgG, IgG1, IgG2, IgG3, and IgG4 respectively. The order of magnitude for the fetal/maternal IgG subclass ratios was IgG1>IgG3>IgG4>IgG2, which resembles the documented order of binding affinity of the human IgG subclasses to placental Fc receptors.

The mean serum levels of all immunoglobulin classes except for IgE were significantly higher (P=0.0001) in HIV-1 seropositive individuals than in HIV seronegative controls. Mean levels in controls were 17.97g/l, 2.07g/l, 1.8g/l, 52.26IU/ml, and 2965IU/ml for IgG, IgA, IgM, IgD, and IgE respectively. All classes, except IgG, showed an increasing trend with evolution of the disease from asymptomatic phase to AIDS phase. The mean IgG subclass levels in controls were 12.76g/l, 1.76g/l, 1.5g/l, and 0.48g/l for IgG1, IgG2, IgG3, and IgG4 respectively. The mean levels of IgG1 and IgG3 were significantly higher in HIV-1 seropositive subjects than in HIV-1 seronegative

controls ($p=0.0001$ for IgG1 and $p<0.0001$ for IgG3). With evolution from asymptomatic to AIDS phase, mean IgG1 and IgG2 levels remained constant, while mean IgG3 level doubled. IgG4 concentration decreased with progression to AIDS phase.

Investigation of anti-HIV-1 IgG subclass response to gp41 and p24 revealed responses in all four subclasses. In the asymptomatic phase, response to gp41 was IgG1 and IgG4 restricted, while response to p24 was mainly IgG1 and IgG3. In the AIDS phase, the IgG subclass response to HIV-1 antigens was IgG1 and IgG2 to gp41, and IgG1 and IgG4 to p24.

Findings obtained in the present study, support the theory of Fc receptor-mediated placental transfer of IgG subclasses, and that perhaps this mechanism is independent of racial and geographical factors. Polyisotypic hypergammaglobulinemia and serum IgG subclass dysbalances were demonstrated in HIV-1 seropositive subjects from an area with mainly heterosexual HIV-1 transmission pattern. The mode of HIV-1 transmission therefore does not appear to influence these phenomena. Isotypic response to gp41 and p24 was demonstrated in all four IgG subclasses. The finding of an IgG3 response to gp41 is of interest in view of the role of IgG3 subclass in the neutralization of viral antigens.

1.0 INTRODUCTION AND REVIEW OF LITERATURE

1.1 INTRODUCTION

Immunoglobulins are the most important molecules produced during the central phase of the humoral immune response (1). They provide the body with a very potent mechanism for dealing with foreign antigens, either by preventing their entry into the body or neutralizing their effect and rendering them less harmful. In so doing, an individual is protected against the deleterious effects of many infectious agents. Apart from their role in the overall immune protection of the host, in the event of an inappropriate humoral immune response, antibodies may cause various immunopathological conditions.

Due to their important role in the immune system, immunoglobulins have been widely studied. Some of these studies have mainly dealt with determination of serum levels in normal as well as in diseased individuals, placental transfer of human antibodies, and antigen-specific antibody responses. Due to variations in normal immunoglobulin concentrations between different races and geographical locations, it is important for each locality to determine its own normal immunoglobulin profiles. Knowing normal levels is a necessary prerequisite for diagnosis of hypo-and hyperimmunoglobulinaemic states. Placental immunoglobulin transfer studies are important in elucidating maternal-fetal humoral immune relationship.

Studies on immunoglobulin profiles in relation to disease states are very important in the understanding of disease-specific immune responses. This is especially important for diseases which profoundly affect the immune system, such as infection with the human

immunodeficiency virus type-1 (HIV-1), first described in 1981 (2). HIV-1 infection being a global public health crisis (3), it deserves a detailed study with regard to its immunological aspects. Knowledge obtained from such studies is of applied use diagnostically, therapeutically and in disease prevention.

1.2 IMMUNOGLOBULINS

The human immunoglobulins are a group of closely related multichain glycoproteins composed of 82-96% protein and 4-18% carbohydrate (4). According to a definition by a special committee of the World Health Organization (5), immunoglobulins refer to proteins of animal origin which have a definite antibody activity, or proteins with similar chemical structure and antigenic specificity. In humans in particular, this group includes proteins exhibiting no antibody activity, for example Bence-Jones proteins.

Immunoglobulins are present throughout the vertebrate kingdom but have not been identified in invertebrates (6) although an IgM-like molecule has been reported in the latter (6,7). All human antibodies are immunoglobulins (7), and these two terms are commonly used synonymously. Immunoglobulins are found in plasma and in other body fluids and tissues, for example in urine, spinal fluid, lymph nodes and the spleen (1,6).

1.2.1 Structure

Porter (8) was the first person to postulate the four-chain structure of immunoglobulins. More detailed examination by Cohen *et al*

(9) supported this structure, which has further been confirmed by immune electron microscopy and x-ray crystallography studies (1,5,6). The basic architecture of all immunoglobulins is now accepted to be the Y-shaped tetrapolypeptide structure elucidated by Porter (8). This is schematically shown in Figure 1.

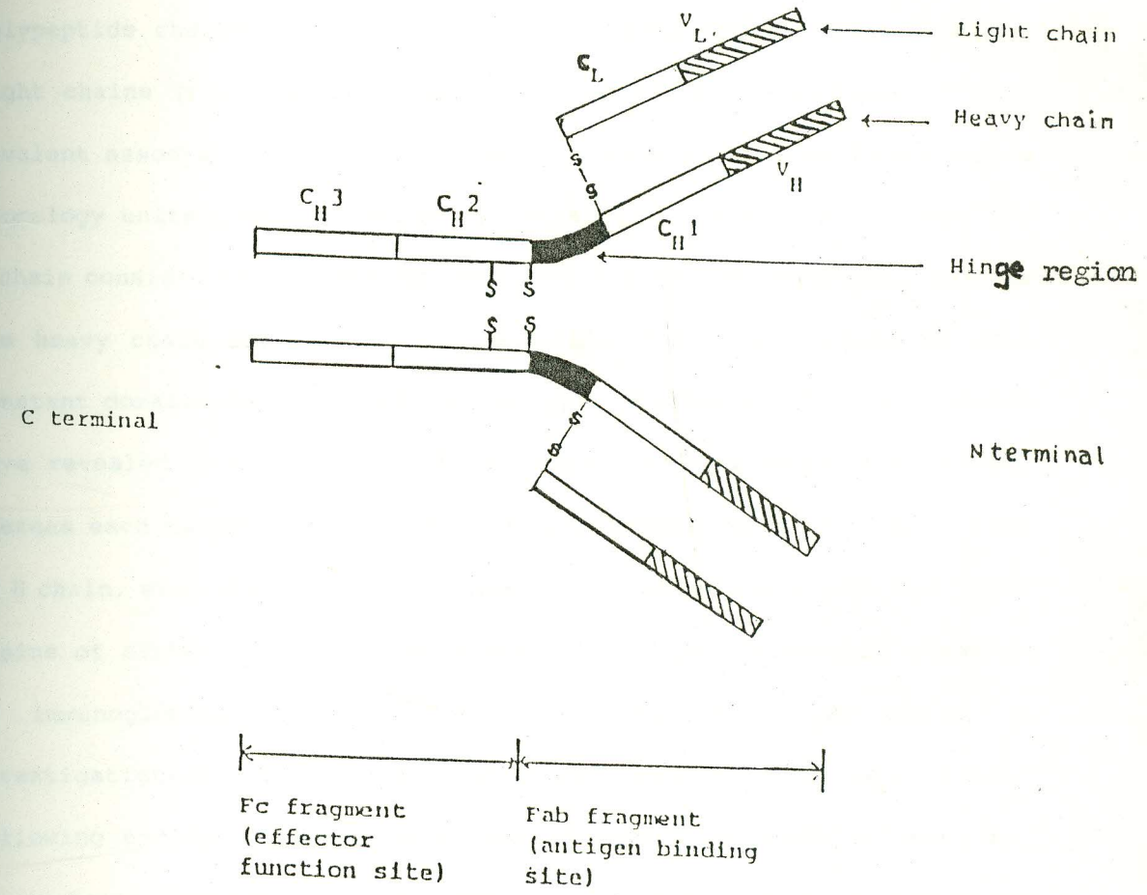


Figure 1: The basic structure of an immunoglobulin molecule

variable region

constant region

hinge region

S-S disulphide linkage

An individual immunoglobulin molecule is composed of four polypeptide chains, two identical heavy chains (H) and two identical light chains (L), held together by disulphide bonds as well as non-covalent associations(1,4). The H and L chains are folded into domains (homology units), held together by intrachain disulphide bridges. The L chain consists of two domains, one variable and one constant, whereas the heavy chain consists of one variable domain and three to five constant domains depending on the isotype. Studies of the light chains have revealed that they are of two types, called kappa and lambda. Whereas each immunoglobulin class is associated with a particular type of H chain, each immunoglobulin molecule, whatever its class, has light chains of either kappa or lambda specificity (6). Enzymatic cleavage of immunoglobulin molecules has been of particular value in investigations of the different biological properties of the molecule. Following earlier work on the action of several proteolytic enzymes, it was found that papain splits rabbit IgG into two identical fragments each with a single combining site for antigen (Fab; fragment antigen binding), and a third fragment termed Fc (fragment crystallizable), so called because it was found to crystallize upon purification (8,10,11). Nisonoff *et al* (10) showed that pepsin digestion of rabbit IgG gives rise to a divalent $F(ab')_2$ fragment and a small C terminal fragment Fc'.

1.2.2 Production

The mechanism by which antibodies are formed has been debated for years. For some time, people believed in the **instructive theory** which

proposed that the specificity of an antibody molecule was determined not by its amino acid sequence but by the molding of the peptide chain around the antigenic determinant. The presence of antigen was required to serve as a template. This theory lost favour when it became apparent that antibody specificity was a function of amino acid sequence and that antibody-forming cells were devoid of antigen.

At present, the **clonal selection theory** is widely accepted. According to this theory, each individual is endowed with a large pool of B lymphocytes, which display immunoglobulin molecules on their surface. These immunoglobulins serve as receptors for a specific antigen, so that each B cell can respond to only one antigen or a closely related group of antigens. When an antigen enters the body, it is phagocytosed, usually by macrophages that process and present the antigen to a B cell that shows the best `fit' by virtue of its immunoglobulin surface receptor. The antigen binds to this receptor, and the B cell is stimulated to divide and form a clone. Some B cells from such clones differentiate into plasma cells and produce antibody which is specific for the antigen which served as the original selecting agent. With most antigens, the induction of an antibody response requires the cooperation of T-helper cells.

Special genetic mechanisms have evolved to produce the very large number of immunoglobulin molecules that develop in the host in response to antigen stimulation, without requiring excessive numbers of genes. In a series of elegant experiments which opened the field of molecular immunology, Tonegawa and coworkers (12) proved that the variable (V) and constant (C) portions of immunoglcbulin were not contiguously

encoded in the germline, but their genes were brought together during the differentiation of the B cell. The V region of each H chain is encoded by 3 gene segments, V, diversity (D), and joining (J) (1, 13). The segments are united into one functional V gene by DNA rearrangement. The V,D,J rearrangement occurs early in ontogeny, is antigen-independent, and is the molecular event that gives rise to the specificity of the immunoglobulin molecule. Each assembled V gene is then transcribed with the appropriate constant (C) gene to produce a messenger RNA (mRNA) that codes for the complete peptide chain. L and H chains are synthesised separately on polysomes and finally assembled in the cytoplasm to form H_2 L_2 units by means of disulphide bonds. The carbohydrate moiety is then added, and the immunoglobulin molecule is released from the cell. Antibody diversity depends on multiple gene segments, their rearrangement into different sequences, the combination of different L and H chains in immunoglobulin assembly, and somatic mutations (1,4).

1.2.3 Electrophoretic pattern

Early studies of antibodies in hyperimmune horse and rabbit sera by Heidelberger *et al* (13) showed that these proteins move to the gamma region on electrophoresis, and were therefore called gammaglobulins. Essentially all proteins in the gamma region are immunoglobulins and this region contains approximately 90% of the circulating immunoglobulins (6). This property of electrophoretic mobility is widely used in various immunoglobulin studies. Figure 2 shows the electrophoretic pattern of serum proteins.

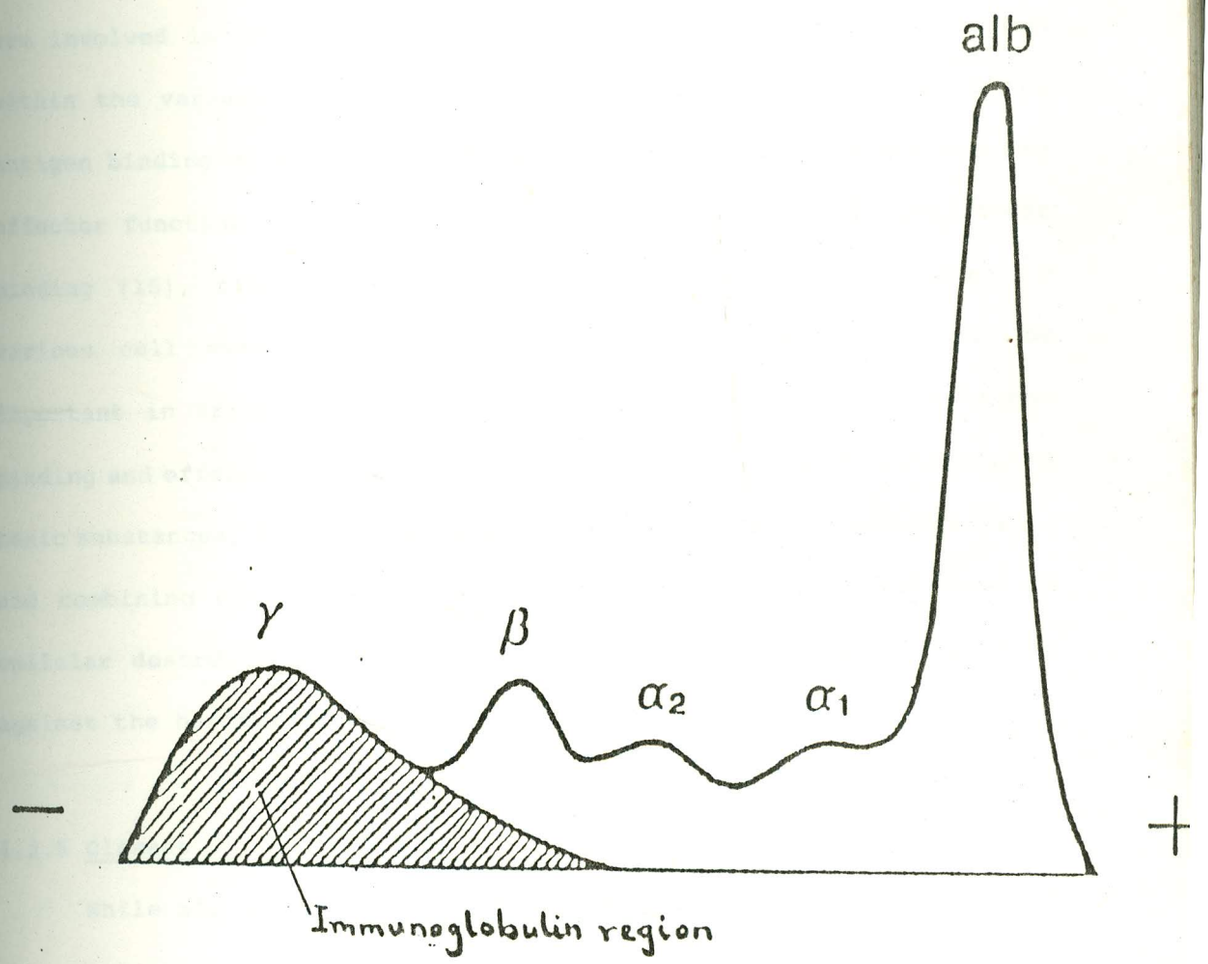


Figure 2. Schematic representation of a densitometric tracing of serum proteins separated by electrophoresis.

1.2.4 Functions

An immunoglobulin molecule has a dual function, namely antigen binding and effector functions. The variable regions of the molecule are involved in antigen binding (6,10). Three hypervariable regions within the variable part of the molecule confer specificity to the antigen binding site (14). The Fc part of the H chain determines the effector function of the immunoglobulin molecule, such as complement binding (15), placental transfer (16,17), and receptor-binding on various cell membranes (6,7). Hinge-dependent flexibility may be important in triggering effector functions (18,19).Through antigen-binding and effector mechanisms, an antibody is capable of neutralizing toxic substances, facilitating phagocytosis and killing microorganisms, and combining with antigens on cell surfaces, which could lead to cellular destruction (1). The net effect is that of host protection against the harmful effects of foreign antigens.

1.2.5 Classes

While all immunoglobulins bear the same basic structure, there are variations of molecular composition and biological function superimposed on their apparent homogeneity (6,7). These variations account for the specificity and biological functions of immunoglobulins, the two properties that characterize them (1,4,6). Based on structural, biological and antigenic differences on the heavy chains, five immunoglobulin classes (isotypes) can be distinguished in man: IgG, IgA, IgM, IgD and IgE (1).

IgG is the most widely studied and best understood of the

immunoglobulins (7). Structurally it is the prototype of all immunoglobulins. It is the major human immunoglobulin, accounting for 12-18% of the serum proteins and approximately 80% of the total serum immunoglobulins (4,6,7). IgG antibodies are mostly, but not exclusively, formed in the secondary or anamnestic response to antigenic stimulus (20,21). IgG plays an important role in the defence against microbial invasions. Owing to its unique ability to cross the placenta (16,17,22), IgG is the most important class that provides immune protection to the newborn in the first months of life.

IgA is the major isotype found in seromucous secretions (1,4,6). In seromucous fluids IgA is present as a dimer, in which two 7S molecules are joined together by a cysteine-rich polypeptide called J-chain. It is stabilized against enzymatic proteolysis by combination with another protein-the secretory component. IgA antibodies prevent entry of microorganisms into the body tissues by inhibiting their adherence to the surface of mucosal cells. Human plasma contains relatively high concentrations of monomeric IgA, whose role remains unclear.

IgM is the first antibody class to appear during the humoral response to an antigen (20), the first to appear in phylogeny (1), and probably the last to leave in senescence (6). IgM molecules are polymers of five four-peptide subunits and is the largest of the immunoglobulins. This class of immunoglobulins is thus referred to as macroglobulin. It is the most efficient isotype for complement activation (1,4). IgM is also found as a membrane receptor on mature B cells (1,4).

IgD is found as a membrane receptor on mature B cells and is present in trace amounts in a normal human serum (1). Very little is known about its biological or structural properties. It has not yet been shown to posses antibody activity, and its function is largely unknown (1,7). However nearly all the IgD is present, together with IgM, on the surface of a portion of blood lymphocytes, where it seems likely that they may operate as mutually interacting antigen receptors for the control of lymphocyte activation and suppression (4).

IgE was first described by Ishizaka *et al* (23) in connection to its role in mediating immediate allergic reactions. This class of immunoglobulins has been shown to posses antibody activity and to fix strongly to tissues; antibodies of the IgE class represent the reagins characteristic of atopic states. Consequently, allergic individuals produce much larger amounts of IgE than do non-atopic persons (23). Furthermore, IgE obtained from the serum of allergic patients is capable of sensitizing the skin of normal recipients to challenge with specific allergens (24). Thus IgE represents a peculiar category of immunoglobulins, which may confer a disadvantage in the form of allergic diatheses of several types. However, it also subserves a beneficial function, for it is known to be involved in the defence against helminthic parasites (1).

The main physicochemical and biological properties of the five immunoglobulin classes are summarised in Tables 1 and 2.

Table 1. Physical properties of major human immunoglobulin classes

Property	IgG	IgA	IgM	IgD	IgE	Ref
Molecular weight	150,000	160,000	900,000	185,000	200,000	1
Sedimentation coefficient	7S	7S,9S,11S	19S	7S	8S	1
Serum levels (Caucasians)**	8-16	1.4-4	0.5-2	0-0.04	0-0.0003	4
Serum levels (Africans)**	14.6-22.5	1.5-3.6	2.1-4.6	*	*	33
Valency	2	2,4	5(10)	2	2	1
% carbohydrate	3	8	12	13	12	1
% Total immunoglobulin	80	13	6	0-1	0.002	1

* Figures not available.

** Levels for adults in g/l.

Table 2. Biological properties of major human immunoglobulin classes

Property	IgG	IgA	IgM	IgD	IgE	Ref
Complement fixation						
Classical	++	-	+++	-		1
Alternative	-	+or-	-	-		1
Placental transfer	+	-	-	-	-	4
Binding to macrophages	+	+or-	-	-	-	1
Fix to homologous mast cells and basophils	-	-	-	-	+	1

+=present, -=absent

1.2.6 Serum levels

The concentration of each immunoglobulin in serum of healthy individuals depends in part on the number of plasma cells that produce that particular immunoglobulin, the rate of synthesis, catabolism and the exchange between intra- and extravascular spaces. Serum immunoglobulin levels have been surveyed in several studies and well documented changes in immunoglobulin concentrations occur during human development (25). It has generally been assumed that changes beyond early adult life are small and of trivial consequence in comparison to those observed during the developmental years. Differences related to race (25,26,27,28,29), sex (25,30) and geographical location (28,29,30)

have been described.

Generally the normal ranges for Caucasians are 8-16 g/l for IgG, 1.4-4 g/l for IgA, 0.5-2 g/l for IgM, 0-0.04 g/l for IgD and 0-0.0003 g/l for IgE (1). In contrast, studies on immunoglobulin profiles in people living in the tropics have shown higher levels of all immunoglobulin classes compared to those of people living in temperate regions (26,27,28,29). In some of those studies, sera from African adults have been shown to have mean levels of the immunoglobulins G, A, and M which are two to three times higher than those in Caucasian control groups (26,29,31). The same magnitude of difference was found in other series comparing Ethiopian and Swedish children of the same age (32). It has been argued that the difference in immunoglobulin pattern between tropical and temperate series is contributed to by a higher prevalence, earlier exposure, and greater severity of various infections in the tropics (26,27,28,29). Nutritional status has also been suggested to explain the racial difference in IgD levels (31) but this claim has not been substantiated.

Studies done in Tanzania (33,34,35) have revealed immunoglobulin values comparable to those found elsewhere in Africa (28,29,30,31). One of these studies (34) also demonstrated the influence of altitude and malaria parasitaemia on the immunoglobulin pattern. In all these studies, quantitations of IgD and IgE isotypes were not reported, and their distribution in the Tanzanian population is unknown.

1.3 SUBCLASSES

Additional antigenic determinants and structural differences

define subclasses. Thus in humans there are four IgG subclasses (IgG1,IgG2,IgG3, and IgG4), (36,37,38); and two for IgA (IgA1 and IgA2), (39,40).

1.3.1 IgG Subclasses

1.3.1.1 Historical perspective

Of the human immunoglobulin subclasses, IgG subclasses are the most widely studied and well characterized. The existence of immunoglobulin subclasses was first noted by Dray in 1960 (36) while conducting studies that defined antigenicity and idiotypes of IgG. This observation was later confirmed by Grey and Kunkel (37), and also by Terry and Fahey (38), who showed that there were four subclasses of IgG namely, IgG1, IgG2, IgG3 and IgG4, representing approximately 65, 25, 6 and 4 percent respectively of the total serum IgG in Caucasian adults (38,41). Since their initial differentiation in 1964, knowledge of the physical, chemical and biological properties of human IgG subclasses has been growing rapidly. The structure of each human IgG subclass protein has been studied extensively, initially with polyclonal antisera rendered monospecific by immunoabsorption, and more recently with monoclonal antibodies.

1.3.1.2 Physicochemical properties

The four human IgG subclasses have more than 95% amino acid homology in their primary sequence (42). Major differences are found in the hinge region in terms of the number of amino acid residues involved and the number of inter-chain disulphide bridges (41,42). The

length and flexibility of the hinge varies with the IgG subclass, and these characteristics influence the biological activities of the subclasses (43). The point of light chain attachement to the heavy chain also differs among the subclasses. IgG1 light chains are bound near the midpoint of the heavy chain, while those of IgG2, IgG3 and IgG4 are joined one quarter the distance from the heavy chain amino termini (41). Both the heavy and light chains contain intrachain disulphide bonds that compactly fold the molecule into domains, which participate in the biological functions of the immunoglobulin. The structural differences between the subclasses are further reflected in their isoelectric points, IgG1 and IgG3 having the highest and IgG4 the lowest. Differences in sensitivity of the IgG subclasses to various proteolytic enzymes has also been reported (41,42,44). Table 3 summarizes the various physicochemical properties of IgG subclasses.

Table 3. Human IgG subclasses: Physicochemical properties

Property	IgG1	IgG2	IgG3	IgG4	Ref
Serum levels:*					
mean g/l	7.1(1.7)	3.0(0.54)	0.65(0.31)	0.6(0.39)	54
proportion %	58.4-71.5	19.4-31.0	4.2-8.4	0.7-6.5	54
Molecular weight	146,000	146,000	170,000	146,000	42
Hinge amino acid no.	15	12	62	12	41
Interchain S-S bonds	2	4	13	2	52
Kappa:lambda ratio	2.4	1.1	1.4	8.0	42
Papain digestion	+	-	++	+	41
Pepsin digestion	+	-	++	-	41
Plasmin digestion	+	-	++	+	41
Trypsin digestion	+	-	+	+	41
Electrophoretic mobility	slow	medium	slow	fast	52

+=susceptible, ++=very susceptible, -=resistant * Adult Caucasians

1.3.1.3 Biological properties

Besides differing in their physicochemical characteristics, the human IgG subclasses manifest a wide variety of different biological properties (41,42,44). Differences in antigen recognition as well as in effector functions have been established. Genetic factors are postulated to explain the differences in response to various antigens as well as the differences in serum levels (44). The differences in effector function such as complement fixation and receptor binding are explained by the differences in primary, secondary and tertiary

structure of the hinge and the constant regions of the heavy chain (41,42).

Placental transfer is one of the important biological properties of IgG subclasses, and has been a subject of several studies (45,46,47). Transfer of IgG1 and IgG3 was first reported by Bernier in 1967 (45). These findings were confirmed by Wang in 1970 (46), who also reported the transfer of IgG4. Wang's study showed a deficient transfer of IgG2, a finding that has been disputed by other workers who have demonstrated transfer of IgG2, though less efficiently than the other subclasses (47). Several documented reports seem to agree that the four subclasses of human IgG are transferable from the mother to the fetus through the placenta (41,42,44,48). Following early observations by Brambell (49) that Fc receptors play a role in the placental transfer of immunoglobulins, several studies have demonstrated the presence of IgG Fc receptors in the human placenta (48,50). Matre and co-workers (51), demonstrated the binding affinity of IgG subclasses to Fc receptors on the human placental membranes to be of the order IgG1=IgG3>IgG4>IgG2. This order appears to suggest that the transfer of IgG across the placenta may be a selective process. However, this theory has not been supported by other studies. For example, the proportions of IgG subclasses in neonatal sera have been shown to be similar to those found in normal adults (45), suggesting that the placental tissue does not play an active role in determining the proportion of IgG subclasses passing into the fetal circulation. On the basis of the finding of a higher IgG level in cord than in corresponding maternal sera, Gitlin (16) and Kohler *et al* (22)

suggested an active transport process as the mechanism for placental transfer of IgG subclasses. This theory has not yet been proved, and the precise mechanism of placental transfer of IgG still remains to be established. Placental transfer of the human IgG subclasses has not been studied adequately in Tanzania.

Complement activation is also another important biological function of IgG subclasses. This takes place through the classical pathway, and is initiated by binding of C_1 to sites on the Fc portion of the IgG subclasses. Activation of complement with the human IgG subclasses varies, and is of the order IgG3>IgG1>IgG2>IgG4 (44). Binding of C_1 to IgG4 is generally not demonstrable, in fact, IgG4 antibodies appear to inhibit immune precipitation and binding of C_1q to IgG1 in complexes containing mixtures of IgG1 and IgG4 (44). IgG4 thus may be considered protective against the biological effects of the complement-fixing antibodies.

Another vital function of human IgG subclasses is their ability to bind to cell surface receptors. Once it is fixed to the surface of certain cell types, an IgG subclass antibody can complex antigen and facilitate clearance of antigens or immune complexes by phagocytosis. Human IgG subclasses are known to bind to other proteins. The Fc region of human IgG1, IgG2 and IgG4 binds to protein A from *Staphylococcus aureus* (1). Inability of IgG3 to bind to protein A is thought to be due to substitution of arginine for histidine at amino acid position 435 (41,42,44). Human rheumatoid factors (RF) react most strongly with IgG1 myeloma proteins followed by IgG2 and IgG4. IgG3 appears to be unreactive with RF (42).

The major biological properties of human IgG subclasses are summarized in table 4.

Table 4. Human IgG subclasses: Biological properties

Property	IgG1	IgG2	IgG3	IgG4	Ref
Complement binding	++	+	+++	-	54
Fc receptor binding(monocytes)	+++	+	+++	+	42
Binding to Staphylococcal protein A	+++	+++	-	+++	41
Reaction with RF	+++	+++	-	+++	1
Blocking IgE binding	-	-	-	+	1
Placenta transfer	++	+	++	++	44
Half life (days)	12-21	12-21	7-8	11-21	54

1.3.1.4 Distribution in normal individuals

The advent of monoclonal antibody technology and development of sensitive assay techniques like enzyme and radio-labelled immunoassays has facilitated quantitation of serum IgG subclasses, and has stimulated interest in measurement of serum concentrations in healthy and in diseased states. In such studies, it has been shown that there is a wide range of serum concentrations for each IgG subclass

(52,53,54). Despite the use of different assay protocols their relative concentrations in serum in Caucasian population has been reported as being 70% for IgG1, 20% for IgG2, 6% for IgG3, and 4% for IgG4. Several factors are known to influence the serum levels of IgG subclasses. Age dependency has been reported by various workers (53,54), who showed that young children have lower immunoglobulin levels than adults, and that the adult level of each subclass is reached at different ages. Serum IgG subclass levels decrease rapidly after birth, reaching their lowest concentration at 3-6 months of age (physiological hypogammaglobulinemia) (42). Thereafter, IgG1 and IgG3 levels rise more quickly towards adult levels than do IgG2 and IgG4 levels. IgG1 and IgG3 reach 50% of adult level by 1 year of age, whereas IgG2 and IgG4 do not reach this level until nearly 4 years of age (53,54). This propensity of the immune system to produce immunoglobulins of the IgG1 and IgG3 subclasses seems to persist even in the very old age group, above 95 years of age (55). Figure 3 shows the development of serum IgG subclasses in children.

Genetic influence on serum IgG subclass levels has been reported (44). The serum levels of some IgG subclasses in Caucasians are thought to be dependent on the Gm type of the individual (44,54). For instance, individuals who are G2m(n+) and G3m(b+) have been shown to have higher serum levels of IgG2 and IgG3 respectively than those who are G2m(n-) and G3m(b-) (44). Thus, the genetic make-up of an individual probably plays a distinctive role in determining the subclass composition of his gammaglobulin (44,54,56). Racial difference in IgG subclass levels has been described (57). The influences of sex and environment have also

been documented (44,54).

Since the IgG subclass levels in serum are influenced by various factors such as age, allotypes, race, sex, and environmental conditions, it is not possible to define universal normal IgG subclass concentrations from the literature. Furthermore, the frequency distribution of all subclasses except for IgG1 are skewed (58), making it inappropriate to use the standard deviation of the mean as a measure of central location. Therefore, normal ranges have sometimes been expressed as the 95th percentile ranges (59). So far, normal ranges for serum levels of IgG subclasses are unknown in many African countries including Tanzania.

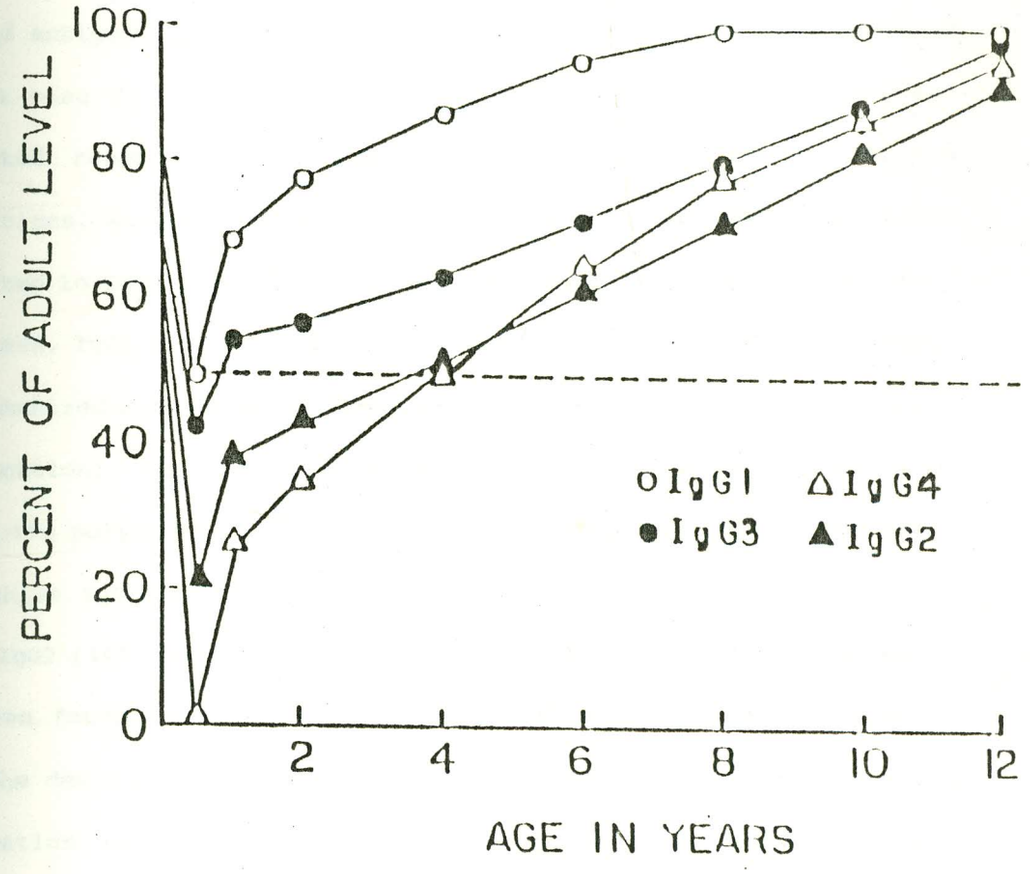


Figure 3. Development of IgG subclasses in children (Data from ref. 53).

1.3.1.5 Subclass responses to various antigens

Elegant observations by Yount *et al* (60) revealed the different IgG subclass composition of antibodies raised in humans against different immunogenic materials. Antibodies to protein antigens tend to be T cell dependent and are predominantly IgG1 and/or IgG3, whereas antibodies to polysaccharide antigens such as bacterial capsules, tend to be T cell independent and are predomindntly IgG2 (54,61,62,63,64). Repeated antigenic challenge with a protein antigen has been reported to give rise to an IgG4 restricted response (65). IgG subclass restricted responses have been observed in relation to various vaccinations. Antibody response to tetanus toxoid has been shown to be restricted to IgG1 (66), although response is also present in all four subclasses. IgG4 restriction has also been demonstrated in individuals hyperimmunized with tetanus toxoid vaccine (Maselle and Matre, personal communication). IgG anticholera vaccine has been demonstrated to be IgG2 to the polysaccharide or teichoic antigens, and IgG4 to the toxin (44), while IgG antibodies to typhoid vaccine have been shown to be mainly IgG2 (44). Anti diphtheria toxoid antibodies, on the other hand, have been found to be associated with the IgG1 and IgG3 subclasses (45). The demonstration of an IgG subclass restricted response during immunisation with various antigens suggests that subclasses are synthesized by the plasma cells independently of each other. However, in this connection, it is important to realise that variations in the IgG subclass behaviour during immune response may possibly reflect differences in the genetic constitution of the individuals concerned (44,54). In addition, factors such as the mode of antigen presentation

and the route of immunisation are also known to play important roles in determining the type of IgG subclass response (44). It is also conceivable that exposure to certain antigenic materials may result in the tolerance of certain clones of plasma cells and the stimulation of others.

1.3.1.6 Subclass responses in various disease states

Selective changes in IgG subclass levels have been noticed in association with infectious and autoimmune diseases, allergic disorders, and immunodeficiency states. IgG1 subclass restriction in viral infections has been reported in rubella (67), cytomegalovirus (CMV) (68), hepatitis B (69), herpes simplex (70), measles (71), Epstein-Barr (72), varicella zoster virus (54) and human immunodeficiency virus type 1 (HIV-1) (73,74,75,76,77,78). Infections with capsulated bacteria such as *Haemophilus influenzae* stimulate IgG2 response (79,80), whereas IgG antibodies to parasitic infections have been found to be restricted to IgG4 in schistosomiasis (81) and IgG1 in malaria (82,83).

Subclass restriction in autoimmune diseases is well documented. Increased levels of IgG1 and IgG3 autoantibodies have been observed in the acute stage of juvenile arthritis, while IgG2 and IgG4 response appears to dominate the picture in patients with partial remission (84). In lupus erythematosus, Sjogren's syndrome, and drug-induced autoimmunity the IgG autoantibodies belong mainly to the IgG1 and IgG3 subclasses (85). Predominance of IgG1 and IgG3 has also been established for the acetylcholine receptor autoantibodies in patients

with myasthenia gravis (86). Antispermatozoal antibodies causing infertility in man have been shown to be restricted to the IgG1 and IgG3 subclasses (44). Anti-thyroglobulin autoantibodies belong to IgG1, IgG2 and IgG3 subclasses (44), while antibodies to glomerular basement membrane in patients with glomerulonephritis are distributed in all four subclasses (54). In allergic disorders IgG4 subclass restriction is the most reported finding (64,87,88). In patients with cystic fibrosis, IgG4 subclass restriction has also been reported (89).

In the majority of these disease conditions, the nature of the disease seems to be related to some unique Fc-located biological activity exhibited by the IgG subclass involved. For instance, IgG4, which is thought to be the only subclass capable of binding to mast cells (41), is frequently elevated in patients with anaphylactic disorders (87,88). On the other hand, antibodies belonging to the complement fixing IgG3 subclass are found in immune complex mediated disorders (54,85).

1.3.1.7 IgG subclass deficiency

Various investigators have reported selective deficiencies of IgG subclasses. Deficiency of one or more of the IgG subclasses may increase susceptibility to recurrent pyogenic infections (90). It is not uncommon to find normal total IgG levels in individuals with IgG subclass deficiency (91), the only hint of a subclass deficiency being the occurrence of chronic bacterial infections (92). It has also been suggested that IgG subclass deficiency may be a common occurrence, and that some of the affected individuals may be asymptomatic (93).

Therefore the precise role of each IgG subclass in the immune response remains to be elucidated. However, as previously discussed, IgG subclass restriction has been shown to occur in response to different types of antigens. Thus, one may postulate that perhaps the dominance of certain subclasses in response to a particular antigen is intended to increase the efficiency of certain effector functions.

1.3.1.8 Quantitative determination of IgG class and subclass levels

Semiquantitative concentrations of IgG subclasses in sera and other body fluids were initially measured by double diffusion in agar (94). In 1965, Mancini *et al* (95) described the use of the technique of single radial immunodiffusion (SRID) in agar in the quantitation of immunoglobulins. Following this development, polyclonal antisera in gel radial immunodiffusion were used for IgG subclass assay. SRID technique was simple to perform, however, criticism of this method focused on the questionable specificity and inter-lot variability of the polyclonal antisera and the limited sensitivity of the SRID for IgG3 and IgG4 (52). The introduction of monoclonal antibody technology has led to the development of more sensitive tests such as radioimmunoassay, enzyme immunoassay, turbidimetry and nephelometry, all of which have provided a tremendous boost in the study of levels of immunoglobulin classes and subclasses in health and in disease states. However, due to the remarkable heterogeneity of immunoglobulins, some monoclonal antisera may not recognize all epitopes of a particular class or subclass (41).

The Human Subcommitte of the International Union of Immunological Societies (IUIS), supported by the WHO, conducted an extensive

collaborative study of 59 monoclonal antibodies with reported subclass specificity by using a variety of immunological assays (96). Following this study highly specific and standardized monoclonal antibodies are now available as research and clinical reagents to facilitate quantitation of each IgG subclass in human serum. Availability of these antibodies has also resolved many of the concerns of specificity and has fostered the development of reproducible immunoassays that can detect minute levels of IgG subclass protein.

1.4 HIV-1 INFECTION AND THE IMMUNE SYSTEM

1.4.1 Historical perspective

The acquired immunodeficiency syndrome (AIDS) was first described in 1981 (2) in the USA. Subsequently, virological and molecular biological studies led to the isolation and characterization of the causative agent (97,98), later called the Human Immunodeficiency Virus type 1 (99). Ever since the first description, HIV-1 infection has become a worldwide epidemic, affecting people in all continents, and has reached such an unprecedented magnitude as to warrant a call for global effort in an attempt to control its spread (3).

The first groups affected by AIDS had as a common denominator, the chance of getting into contact with blood or blood products through sharing of syringes and needles (100), repeated transfusions (101,102) and homosexual practice (103). With further spread of HIV-1 infection, heterosexual transmission emerged, and this mode of spread is now reckoned to be the commonest mode of HIV-1 transmission in Africa (104,105,106).

1.4.2 Agent:Morphology, molecular organisation and antigenic structure

1.4.2.1 Morphology

HIV-1 is an enveloped RNA virus which displays morphological similarities with the other lentivirus members (107). Mature HIV-1 particles have a diameter of about 100nm, and contain a bar-shaped central nucleoid (108). Each virion has two molecules of identical single-stranded RNA, and an RNA-dependent DNA polymerase (reverse transcriptase) which is tightly associated with the genetic material (108). The capsid has icosahedral symmetry and a matrix protein lines the internal part of the lipid membrane. The envelope is composed of glycoproteins.

1.4.2.2 Molecular organisation and antigenic structure

Like other retroviruses, the genome of HIV-1 has one gene for the envelope glycoproteins **(env)**,one gene for the viral internal core proteins **(gag)**, and one gene for the reverse transcriptase or polymerase **(pol)** (109). In addition to these structural genes, HIV-1 contains several regulatory genes for the control of viral replication and for viral infectivity (107). The genetic complexity and high degree of genetic variability seen in HIV-1 is reckoned to be unprecedented among retroviruses.

1.4.2.2a The structural HIV-1 genes and their encoded proteins

Mature structural and enzymatic proteins of HIV-1 arise from the proteolytic processing of large precursor proteins (110).

gag: This gene encodes the matrix protein p19, the core protein p24 and

two small polypeptides p7 and p9 which are found associated with the virus genetic material in the virion core. Antibodies to **gag** proteins develop early during seroconversion and are present during the asymptomatic period of HIV-1 infection (111). Concomitant with the appearance of HIV-1 antigenaemia, there is a decline of antibody to the viral core protein p24, and this has been associated with disease progression (112).

pol: This gene encodes the HIV-1 enzymatic proteins, which include protease, reverse transcriptase, ribonuclease H and integrase (endonuclease), denoted p10, p66, p15, and p31 respectively (113). Loss of enzyme activity as well as viral infectivity occurs following mutations in critical regions of polymerase (114). This observation is of applied value in that drug therapy can be targeted towards inducing mutations in the polymerase. Ribonuclease H is a cleavage product of p66 and degrades the viral RNA strand of the initial RNA:DNA hybrid in the replication process (115). Endonuclease is responsible for integration of proviral DNA into the cell genome (115). Majority of HIV-1 infected individuals have precipitating antibodies to endonuclease and reverse transcriptase products (116).

env: The envelope gene encodes for gp160 which is cleaved into a glycosylated hydrophilic protein gp120 and transmembrane protein gp41. Glycoprotein 120 shows the most extensive variability among the HIV-1 proteins (117). Envelope proteins play a central role in receptor binding, induction of cytopathic effect and development of humoral and cellular immune responses (111,118). Antibodies to the envelope proteins are the first to appear during seroconversion (111) and are

detectable throughout the entire course of the disease. Some functional and immunogenic envelope sites have been identified, and these include neutralization site (119), (CD4) binding site (120) and an antibody dependent cell mediated cytotoxicity (ADCC) target (121) on gp120; fusogenic site (122) and antigenic site (123) on gp41.

1.4.2.2b HIV-1 regulatory genes

HIV-1 encodes several regulatory proteins which control the level of virus production through a sophisticated network of yet poorly understood interactions (107). The HIV-1 regulatory elements appear to be involved in the maintenance of persistence of activation (124).

1.4.3 Pathogenesis of HIV-1 infection

1.4.3.1 Mechanism of infection

Selective tropism of HIV-1 for the T4 cell was noted in the very early reports of AIDS (2). Following isolation of the aetiological agent of AIDS it was subsequently established that the T cell membrane bound protein CD4, used to identify the T-helper cells, was also the binding site of the virus (118,125). Further experiments utilizing restriction fragments of viral DNA to produce recombinant mutants of the viral envelope have demonstrated that binding occurs between the gp 120 envelope glycoprotein and the CD4 receptor (126), in association with T cell surface expressed HLA-DR class II histocompatibility antigen (127). The role of CD4 molecule as HIV-1 receptor is now firmly established, evidence for this being the selective replication of HIV-1 in CD4+ cells, the blocking of

infectivity by anti-CD4 antibodies, infection of HeLa cells possible only after insertion of the CD4 gene, and co-precipitation of the CD4-gp120 complex (128). The Fc receptor has been shown to bind an antibody-HIV-1 virus complex *in vitro*, and in this way allow entry of HIV-1 virus into hitherto CD4 negative cells (129). Entry of HIV-1 into certain cells, for instance in the central nervous system, may also be via an alternative receptor (130), however, evidence for this is still scanty. Following infection and replication, a wide range of immunological abnormalities have been described.

1.4.3.2 Immunological abnormalities

1.4.3.2a Defects in cellular immunity

The T4 lymphocyte is the critical cell involved directly or indirectly in the induction of most immunologic functions (1,4), which include sending inductive signals to other T cells, Natural Killer (NK) cells, B lymphocytes and some non lymphoid cells. Due to its important role in mediating immune responses, quantitative deficiency or functional impairment of the T-helper subset by HIV-1 infection results in a profound immunological derangement (128,131,132,133).

Quantitative deficiency is the most conspicuous T4 cell defect in HIV-1 infection (132,133,134). Direct viral cytopathic effect has been proposed to be the cause of the quantitative T4 deficiency but the precise mechanism for this cytopathicity is largely unknown (128). Accumulation of unintegrated viral DNA has also been thought to have a direct cytopathic effect (132). Another mechanism that has been suggested is that of a massive increase in permeability of the cell

membrane when large amounts of virus are produced and bud off the cell surface (128). However, the number of HIV-1 infected T4 cells is usually small as demonstrated by studies involving *in situ* hybridization with HIV-1 probes, which have demonstrated that only a small percentage of T4 cells in the peripheral blood of HIV-1 infected individuals express viral proteins (132,133). These findings appear to suggest that T4 cell depletion may also be a result of mechanisms other than direct viral cytopathic effect. Several such mechanisms have been proposed, which include syncytia formation (135,136), and direct killing of non-infected T4 lymphocytes coated with free gp120 by cytotoxic T cells or through antibody dependent cell mediated cytotoxicity (133,134). It has also been proposed that HIV-1 infected cells can undergo changes in their HLA class II phenotype and become susceptible to an autoimmune attack (128). The possibility of HIV-1 infection inducing production of soluble substances toxic for T4 cells has also been raised (133) but is yet to be proved. Formation of complexes of CD4 protein and viral envelope proteins inside T4 cells may also lead to their destruction (131,133). Another possible mechanism for T4 destruction is that HIV-1 infected lymphocytes may become susceptible to superinfection by other pathogens which enhance viral cytopathicity. This is probably achieved through the action of HIV-1 transactivator genes, which cause productive infection of the cell leading to cell death (137). In support of this hypothesis is the *in vitro* observation that CMV can only cause abortive infection in uninfected T cells, but causes a productive infection in HIV-1-infected T cells (137). Due to the decreased number of T4 cells, the CD4/CD8

ratio is reduced (132).

Depletion of T4 lymphocytes is the most obvious immunologic abnormality observed in HIV-1 infected individuals. However, a functional impairment of antigen responsive T4 cells is also a common finding. Studies involving stimulation of peripheral blood mononuclear cells with common mitogens have revealed the selective deficiency of antigen responsive T cells early in the course of AIDS (138), in the absence of any abnormality of antigen-presenting cells required for such responses. Several possibilities have been raised to explain the functional T4 cell defect. One such mechanism is that HIV-1 or its products may impair the signal transduction process of T4 cell response to antigen (139). HIV-1 envelope proteins may also block antigen specific responses by interfering with the normal interaction between class II major histocompatibility complex molecule on antigen presenting cells and the CD4 molecule on T4 cells (133,140). Experimental studies have also shown that some HIV-1 infected T4 cells fail to express CD4 molecules on their surfaces. Other workers have demonstrated a decreased expression of interleukin 2 (IL-2) gene which is critical to many T cell functions (128).

Although infection of monocytes or macrophages by HIV-1 causes a negligible cytopathic effect, functional defects such as defective chemotaxis have been reported (141). The phagocytic function, and antigen processing and presentation by HIV-1-infected monocytes and macrophages is thus impaired. Involvement of alveolar macrophages leading to impaired phagocytosis is thought to explain the high incidence of *Pneumocystis carinii* pneumonia in patients with AIDS

(133). The occurrence of fevers and wasting syndromes in AIDS patients has been attributed to increased secretion of monokines such as IL-1 and tumour necrosis factor (128). An important implication of the infection of monocytes with HIV-1 is the possibility that the monocyte may serve as a reservoir for HIV-1 in the body (128,131), and could serve as a transport cell to various organs in the body such as the lung and the brain. Defective NK cytotoxicity has also been demonstrated (139).

1.4.3.2b Abnormalities of the humoral immune response

The scope of immune dysfunction in AIDS is now known to involve B lymphocytes, and this is manifested by hypergammaglobulinemia, circulating immune complexes and autoantibodies (131,142). B lymphocyte function is significantly impaired following infection with HIV-1 (142). In the early phase of infection, there is marked hypergammaglobulinemia which results from polyclonal B cell activation (142,143). The mechanism behind polyclonal activation is not fully established but multiple factors have been considered to be responsible for this phenomenon. It has been suggested that HIV-1 or various HIV-1 proteins could act as B cell mitogens and hyperstimulate the B cells (144). There are reports in literature which suggest that B cells may be infected with HIV-1 both *in vivo* and *in vitro* (145,146). Direct infection of B cells with HIV-1 could then stimulate polyclonal antibody production. Immunoglobulin overproduction could also result from defective regulation of T suppressor cells, thus permitting uncontrolled immunoglobulin production by B cells (147). It has also

been proposed that the phenomenon of polyclonal B cell activation may result from latent infection with polyclonal B cell activating viruses, such as Epstein-Barr virus and CMV, both of which are prevalent in HIV-1 infected individuals (134). HIV-1 is also capable of directly enhancing B cell growth and differentiation, giving rise to increased number of immunoglobulin secreting cells (132). However it remains unclear if this results in polyclonal B cell activation (132). Recently, a putative polyclonal B-cell stimulatory epitope has been demonstrated in the carboxyl end of the envelope glycoprotein of HIV-1 (148).

In sera from homosexual individuals, the common immunoglobulin isotypes involved in polyclonal B cell activation are IgG, IgA and IgD (143). IgD elevation occurs early in HIV-1 infection (149), reaches a peak in patients with AIDS-related complex (ARC) and drops to lower levels in patients with AIDS. However, the IgD levels in patients with AIDS remain elevated as compared to normal controls. The mode of HIV-1 transmission has been postulated to influence the pattern of serum hyperimmunoglobulinaemia (74,143), but this has not been conclusively verified.

Serum immunoglobulin G subclass dysbalances have been described in HIV-1 infection (75,76,77). In these studies, elevation of serum levels of IgG1 and IgG3 and decreased IgG2 concentration in HIV-1 infected patients has been demonstrated. The pattern of IgG1 and IgG2 subclass-specific immunocytes in lymphnodes of HIV-1 infected patients has also been shown to correlate with the serum pattern of IgG1 and IgG2 subclasses (76).

Despite the heightened activity of B cells, they are impaired in their ability to respond to specific antigenic stimulation (142). This may be partly explained by defects in T-helper cell function. However, other mechanisms remain to be defined. Particularly evident is the inability of B cells to mount an IgM response to antigenic challenge (142). IgM response is important for the initial defence against infectious agents. Frequent pyogenic infection in HIV-1 infected children could thus be due to failure of defective B cells in producing antibodies of the IgM isotype (132).

1.4.4 The host immune response in HIV-1 infection

1.4.4.1 Humoral response

Several studies have shown that HIV-1 infected individuals with or without AIDS develop antibodies against the structural HIV-1 proteins (111,112,116). Antibody responses to the viral core proteins and envelope glycoproteins occur early, within 4-12 weeks of exposure to HIV-1 (111). Despite the presence of high titer antibody to core protein p24, the neutralizing activity of this antibody has been observed to be low (111,112). It has also been observed that p24 antibody levels decline in patients with AIDS (112). Studies on antibody response to HIV-1 proteins have demonstrated an isotypic restriction (74), in which responses to **gag**-products involve IgM,IgG1,IgG3 and IgA. Response to **env** proteins is restricted to the IgG1 isotype while response to regulatory protein F is restricted to IgM, IgG, and IgA. On the basis of these observations, it has been suggested that there may be different regulatory mechanisms in the B

cell response to the various HIV-1 proteins (74). It is well established that the four human IgG subclasses are produced in response to various types of antigens (60). Protein antigens like those of viruses induce IgG1 and IgG3 response (60,68,69,70), while IgG2 is the predominant response to bacterial polysaccharides (79). Repeated antigenic stimulation by viruses has been shown to induce an IgG4 response (88). In HIV-1 infection, IgG1 has been shown to be the dominant antiviral subclass in healthy seropositive individuals (74,78). IgG3 is detected mainly in individuals with recent infection (73,78). Anti-viral IgG3 is of special interest due to its high neutralizing capacity for other viruses where it is directed to envelope proteins (150). In HIV-1 infection IgG3 response is directed to **gag** proteins, and does not appear to have neutralizing capacity. Utilizing molecular biological techniques, investigators have been able to detect conserved sequences within the gp120 which stimulate production of viral neutralizing antibody (134), but IgG subclass restriction to this conserved epitope is not yet documented. The significance of these antibodies in limiting the viral infection directly or as markers for vaccine candidates remains to be determined. Loss of IgG3 and IgG4 subclasses has also been correlated to progression of clinical symptoms (74,151,152), an observation which could be of prognostic significance. Studies on at-risk individuals have revealed the presence of **gag** and **pol**-specific IgM or IgA in the absence of specific IgG isotypes (74). In such individuals, presence of **gag** and F-specific antibodies has been demonstrated in the absence of **env**-specific antibodies (74). These observations suggest that

isotypic and antigenic selection mechanisms occur early in the course of HIV-1 infection, and are therefore of potential diagnostic interest.

Some studies have demonstrated that some HIV-1-seropositive individuals make anti-viral antibodies which can cross-react with an antigenic epitope within the CH1 domain of their own IgG (153), and an autoimmune reaction has thus been implied. Whether this plays any significant role in the immunopathogenesis of HIV-1 infection remains to be established. Antibody-dependent enhancement of infection with HIV-1 has been reported (154), and is thought to be one of the pointers of bad prognosis.

1.4.4.2 Cellular immune response

The role of cellular cytotoxicity in controlling HIV-1 infection has been investigated. Antibodies in the sera of HIV-1-infected individuals that are capable of participating in ADCC have been described (155,156,157). These antibodies are mainly directed to gp120 (155). However, some workers have demonstrated a high degree of correlation between antibody reactivity to p24 and the ability to direct an anti-HIV-1 ADCC (157). ADCC activity in HIV-1 infection declines with progression of infection (156,157). As well as being protective, the phenomenon of ADCC could conceivably accelerate disease progression via non infectious lympholysis. This may occur when free gp120 binds to CD4 molecules on uninfected lymphocytes, rendering them susceptible to destruction via ADCC (155). Although the role of ADCC in HIV-1 infection remains uncertain, this form of cytolytic reactivity could be beneficial to individuals in early infection. Antibodies that

mediate ADCC may remove from the circulation, cells infected with a wide variety of viruses, thus proving to be a valuable adjunct to neutralizing antibodies in vaccines. Antiviral suppressor response has also been demonstrated. T lymphocytes bearing the CD8 marker from HIV-1 infected individuals have been shown to suppress viral reproduction in the patients own CD4 T cells (158). Other workers have demonstrated the presence of cytotoxic lymphocytes bearing CD3 and CD8 surface markers in the blood of HIV-1 infected individuals. This lymphocytotoxicity is reported to be specific for HIV-1 reverse transcriptase (159). This finding could be of importance in vaccine development, since the gene that encodes HIV-1 reverse transcriptase is highly conserved among different HIV-1 strains (159).

1.4.5 Immunological tests for HIV-1 infection

Since the successful isolation and culture of HIV-1 in permissive cells (98) and the demonstration of serum antibodies to HIV-1 proteins (111), several immunological approaches to HIV-1 testing have been established.

1.4.5.1 Antibody detection

Majority of tests for HIV-1 infection are based on detection of anti HIV-1 antibodies (160). Several techniques have been devised, and these include enzyme and radio-labelled immunoassays, fluorescent antibody tests, and immunoblotting. Of these, enzyme immunoassays are highly sensitive, easy to perform, and are more cost-effective than most other tests (161). However, the high sensitivity may compromise

their specificity, giving rise to a number of false-positive results. This is attributable to non-specific binding antibodies which are sometimes found in body fluids. This aspect is of a particular interest in HIV-1 serology in the tropics where the presence of a variety of microbial infections may give rise to cross-reacting antibodies. Enzyme immunoassays are very useful in HIV-1 screening of blood and blood products. Immunoblotting, particularly the western blot technique is used as a confirmatory test. Radioimmunoprecipitation and indirect fluorescent antibody tests are used mainly as research tools, although to a limited extent, they have also been used as confirmatory tests. Some rapid tests based on passive agglutination technique have also been used. Detection of antibodies to specific HIV-1 proteins such as p24 may be used in monitoring disease progress, a decrease in anti-p24 antibody titer being an indicator of worsening prognosis.

1.4.5.2 Antigen detection

Several tests based on HIV-1 antigen assay are now available, and include enzyme immunoassays, competitive radioimmunoassays and indirect immunofluorescence assay methods (161). The HIV-1 antigen assay has improved the laboratory diagnosis of HIV-1 infection by providing a more sensitive marker than routine antibody screening during the early stages of infection, and for confirming neonatal infection in the presence of passively transferred maternal anti-HIV-1 IgG (161). It is useful in the diagnosis of persistent infection in children who remain infected but are seronegative. In addition, HIV-1 antigen assay has led to improvement in virus detection in culture instead of the less

sensitive and more time-consuming reverse transcriptase assay. Antigen assays have also been used in monitoring HIV-1 disease progression, whereby an increasing antigen titer is correlated with the progression of disease.

2.0 RATIONALE OF THE STUDY

It is evident from this review that quantitation of serum immunoglobulins is important in elucidating humoral immune response patterns both in healthy and in diseased states. Knowledge of the normal immunoglobulin profile is important in establishing diagnosis of immunopathological conditions. Since serum immunoglobulin levels in people who live in the tropics are reported to be higher than those of people living in temperate regions (30,31,32,33), it is necessary for each locality to determine its own immunoglobulin profiles for a rational evaluation of immunological disorders characteristic in that particular environment. Previous studies on immunoglobulin concentrations in Tanzania have mainly dealt with quantitation of IgG, IgA and IgM isotypes (32,33), while levels of IgD, IgE and IgG sublcasses remain largely unknown.

Study of immunoglobulin profiles in maternal and cord sera is important in the understanding of maternal-fetal humoral immune relations, especially the aspect of transplacental transfer of antibodies. Presence or absence of placental transfer of a particular immunoglobulin isotype can be ascertained, and such an information may be of applied value in establishing diagnosis of vertically-transmitted

be of applied value in establishing diagnosis of vertically-transmitted infections. It is of interest to examine the fetal-maternal IgG subclass ratios as these have not been studied adequately in Tanzania.

Analysis of immunoglobulin levels in HIV-1-infected individuals may be of diagnostic importance and is also of value in monitoring progression of the disease. Studies on isotypic response to specific HIV-1 antigens are of immense value in vaccine development and trials. Little is known of the immunoglobulin profiles in HIV-1-infected Tanzanians, and to the best of the author's knowledge, there is no documented study on IgG subclass response to HIV-1 antigens in Tanzania. Since HIV-1 infection is a public health problem in Tanzania, this kind of a study seems justified. The mode of HIV-1 transmission has also been thought to influence the isotypic restriction seen in the hyperimmunoglobulinaemia associated with HIV-1 infection. Since previous studies have mainly dealt with individuals infected through homosexual practices and parenteral route, it is of interest to investigate such a phenomenon in a region where heterosexual transmission pattern predominates.

In addition, this study will acquaint the author with the various immunological techniques involved in immunoglobulin quantitation, as well as cultivating a sense of scientific inquiry and critique.

3.0 OBJECTIVES

3.1 Broad objective

To study the immunoglobulin isotype profiles in healthy and in HIV-1 infected individuals, and the IgG subclass response to defined envelope and core HIV-1 antigens in Dar es Salaam.

3.2 Specific objectives

1. To determine the serum levels of immunoglobulin classes and IgG subclasses in healthy pregnant mothers and their newborns.

2. To determine the serum levels of immunoglobulin classes and IgG subclasses in healthy HIV-1 seronegative individuals.

3. To determine the serum levels of immunoglobulin classes and IgG subclasses in HIV-1-seropositive individuals.

4. To investigate the IgG subclass response to gp41 and p24 HIV-1 antigens in sera from HIV-1 seropositive asymptomatic individuals and in patients with AIDS.

4.0 MATERIALS AND METHODS

4.1 Subjects

These were drawn from individuals attending different departments at Muhimbili Medical Centre between July and August 1989, and comprised of:

1. 40 full term mothers (mean age 25.4; range 17-39 years) seen in the labour ward, who had no history of any infection in the past two weeks prior to the specimen collection. They also had no medical or obstetric complications from history and clinical examination as well as from the records shown in their antenatal cards. In order to exclude individuals who might have been infected during labour, only those mothers whose membranes had ruptured in less than four hours were included in the study. Advantage was taken of an ongoing study on vertical transmission of HIV-1 infection, to select only those mothers who were HIV-1 seronegative. All their respective neonates were included in the study.
2. 80 clinically healthy blood donors (mean age 31; range 20-56 years) seen in the blood bank, 30 of whom were HIV-1 seropositive and 50 HIV-1 seronegative. These two groups were matched for age and sex.
3. 20 patients (mean age 33; range 21-56 years) with symptomatic HIV-1 infection, admitted in the medical ward and classified as having AIDS according to the clinical case definition as defined in the Bangui criteria (162).

In order to avoid any racial bias in the immunoglobulin pattern, the

study subjects comprised of individuals of African origin only. Specimen collection was done from 1st July 1989 to 30th August 1989 in Dar es Salaam while laboratory studies were carried out in Bergen from March 1990 to August 1990.

4.2 Serum samples

Maternal blood was collected by venepuncture under aseptic precautions, and centrifuged after clotting to obtain sera. Cord blood was obtained at delivery and processed in the same way to obtain serum. Some blood from each donor was put in a universal container, from which serum was obtained after clotting. Sera from patients with AIDS were obtained in the same manner as those from pregnant mothers. After collection, all sera were aliquoted and stored at -20°C. The sera were later transported, in a cold box with ice cubes, from Dar Es Salaam to Bergen where they were stored at -20°C until the time for assay.

4.3 HIV-1 serology

All sera were screened for HIV-1 infection by two ELISA tests: Organon anti-HTLV-III (Organon teknika) and Wellcozyme recombinant (Wellcome Diagnostics, England). Sera with reactive ELISA in both tests were confirmed with western blot (DuPont HIV-1 Western Blot kit). Western blot seropositivity was based on the WHO criteria for the interpretation of HIV-1 western blots (163); ie. a strip was considered positive only when there were definite bands for two envelope proteins plus or minus one core protein.

4.4 Estimation of C-reactive protein (CRP)

This was done for all maternal sera and sera from HIV-1 seronegative blood donors in order to exclude the presence of acute infection. A latex agglutination test was used. The test was done according to the manufacturer's instructions (Rapi Tex-CRP, Behringwerke, West Germany). A drop of CRP reagent was mixed with a drop of test serum on a glass slide. A positive control serum was also mixed with the CRP reagent. Results were read within two minutes. Results were interpreted as positive when there was agglutination in the test serum within two minutes, and negative when agglutination was not detected. According to the manufacturer's instructions, the test is positive when the level of C-reactive protein in the test sample is more than 6mg/l.

4.5 Quantitation of IgG, IgA and IgM

This was done by using a laser nephelometer (Behringwerke AG), which uses the principle of immunoprecipitation for antibody assay. The machine was first calibrated using standard antisera for human IgG, IgA and IgM (Behring Lot. No. 153058 D, 153161 D and 153277 A respectively). N/T protein, a product recommended by the manufacturer, was used as a control. Reference curves were drawn for each standard. Throughout the running, a phosphate buffer saline with sodium azide was used as a buffer.

4.6 Quantitation of IgD and IgE

This was done by single radial immunodiffusion technique as described by Mancini *et al.* (95). LC-Partigen^{R} immunodiffusion plates

(Behringwerke AG, Marburg,W.Germany) were used. These contained the specific antiserum to either IgD or IgE in an agar-gel layer ready for use. The assay protocol adopted was that recommended by the manufacturer. During each assay, a plate was opened and left to stand for 5 minutes at room temperature for evaporation of any water of condensation that may have entered the wells. Three wells were filled with 20 microliters of the suitable standard dilutions (3 different concentrations). For IgD assay, the standard was human serum (Lot No.046417A Behringwerke AG) in 1:4, 1:2 and 1:1 dilutions, corresponding to concentrations of 47, 95, and 188 IU/ml respectively. For IgE assay, a standard human serum (Lot No.046516B Behringwerke AG) in dilutions of 1:4, 1:2 and 1:1 corresponding to concentrations of 1940, 3880, and 7760 IU/ml respectively was used. Twenty microliters of each test sample were filled in the remaining wells. For IgE assay, another 20 microliters were added into the wells 30 minutes after the initial 20 microliters. All assays were done in duplicate. After loading, the plates were allowed to stand open for 10 minutes, then closed with a plastic lid and left to stand at room temperature for 2 days in the case of IgD and 5 days for IgE. Precipitin ring size was measured with a Mebprojektor ocular (Behring institut) to the nearest 1/10th of a millimetre. Final diameters were obtained by averaging the duplicate readings. Using a linear graph paper the squares of the diameters of the precipitate rings obtained with the standard dilutions were plotted as a function of the immunoglobulin concentration (abscissa: antigen concentration ; ordinate: squares of the ring diameter in mm^2). Unknowns were then read off the standard curve. The

assay ranges for the immunodiffusion plates as given by the manufacturer, were 14.0-222IU/ml for IgD and 920-11000IU/ml for IgE. For the IgD and IgE values below the lower limit of assay detection, they were assigned values midway between 0 and the lower limit of the assay in order to avoid a disproportionate impact of these values on subsequent calculations.

4.7 IgG subclass assay

An immunoenzymatic microtitre method based on a `sandwich' enzyme immunoassay technique was used. Kits of microtitre strips with wells pre-coated with different monoclonal antibodies specific for the relevant IgG subclass (from The Binding Site limited, Birmingham United Kingdom) were used. During each assay, the kit was removed from storage and allowed to warm to room temperature for 30 minutes before use. During each assay, a kit control and a WHO human IgG subclass control 67/97 (164) were included. Seven dilutions of a standard serum (included in the kit) were assayed on the plate for the calibration of a standard curve. Test samples were diluted according to the manufacturer's instructions. One hundred microlitres of each sample were dispensed in duplicate into each well of the microwell strips. The wells were covered with a plate lid, placed in a moist box to prevent drying, and incubated at 37°C for two hours. The wells were then washed three times using 200 microliters of a working strength wash buffer provided in the kit. One hundred microlitres of conjugate (sheep anti-human IgG conjugated with horseradish peroxidase enzyme) were dispensed into each well, and the plates were covered, placed in the moist box

and incubated at 37°C for two hours. After the conjugate incubation washing of the wells was done three times as previously. One hundred microlitres of substrate solution (orthophenylene-diamine in buffered hydrogen peroxide solution) were dispensed into each well as quickly as possible in order to avoid assay drift. The plates were then left at room temperature for 30 minutes, after which the reaction was terminated by 50 microliters of a stop buffer (2.5M H_2SO_4) into each well. The optical density (OD) of each well was read at 450nm on a microwell plate reader (Organon Teknika 510) immediately after the procedure.

Due to lack of enough commercially precoated assay strips, 12 maternal and the corresponding cord sera were assayed by using serocluster U vinyl ELISA plates (Costar, Cambridge, UK) coated with monoclonal mouse antibodies to the IgG subclasses, according to the method described by Hamilton *et al* (165). The monoclonal antibodies used were anti-IgG1 (HP6012, Oxoid, UK), anti-IgG2 (HP6009, Oxoid,UK), anti-IgG3 (HP 6047, CALBIOCHEM ,Behring diagnostics La Jolla, California), and anti-IgG4 (HP 6023, CALBIOCHEM, Behring diagnostics). One hundred microlitres of the monoclonal antibodies were dispensed into each well and the plates were then incubated at 37°C for 2 hours, then stored at 4°C overnight. The following day, the wells were washed three times with phosphate buffer saline (PBS). A hundred microlitres of 1% bovine serum albumin were dispensed into each well for blocking any uncoated sites, followed by incubation at 37°C for 1 hour, after which the plates were again washed with PBS. IgG subclass assay was then done using the same procedure as for the commercially precoated

strips

For each IgG subclass assay, a standard curve was constructed by plotting the mean OD of the standard dilutions against the corresponding IgG subclass concentrations. The mean OD of the diluted control was used to read the concentration of the diluted control from the curve. This value was then multiplied by the dilution factor to obtain the concentration of the undiluted control sample. A curve was considered to be correct when the values of the controls read from the curve were similar to the ones given for the kit control and the WHO standard preparation. Subclass levels in each test sample were calculated by multiplying the value obtained from the curve and the dilution factor.

4.8 Assay of IgG subclass reactivity to gp41 and p24

An ENVACOR HIV-1 enzyme immunoassay kit (ABBOTT Diagnostics) was used. This kit had separate reaction trays for ENV and CORE antigens. In the assay procedure, one ENV HIV-1 (rDNA) coated bead (for gp41 assay) or one CORE HIV-1 (rDNA) coated bead (for p24 assay) was added to each reaction well. Test sera (100 microliters) were dispensed into the reaction wells in optimum dilutions as earlier pre-determined in a checkerboard titration. In each assay negative and positive control sera were included. The trays were incubated for 2 hours at 37°C, followed by washing of the beads three times using double distilled water. Two hundred microliters of mouse monoclonal antibody appropriate for each IgG subclass; ie. IgG1 (HP6012, Oxoid, UK), IgG2 (HP6009, Oxoid UK), IgG3 (HP 6047, CALBIOCHEM,Behring diagnostics, La

Jolla,California) and IgG4 (HP 6023, CALBIOCHEM) were added into each well followed by 2 hours incubation as previously. The beads were again washed as before and 200 microliters of horse raddish peroxidase conjugated rabbit anti-mouse IgG (1/500 dilution in PBS containing 10% pooled human serum) were dispensed into each well. The trays were incubated for 2 hours at 37°C, followed by washing of the beads as previously. The beads were then transfered each into a separate assay tube. Three hundred microliters of freshly prepared OPD substrate solution were added into each tube, and left at room temperature for 30 minutes. Reaction was terminated by 1ml of 1N sulphuric acid and the intensity of the colour developed was read using Quantum analyzer (Abbott diagnostics) set at 492 nm. The cut off value was obtained by dividing the sum of the OD for the positive and negative controls by 2. Samples with absorbances greater than the cut off value were considered positive for IgG subclass antibody to HIV-1, and values less than the cut off value were considered negative.

4.9 Statistical analysis

Results obtained were analyzed with matched Student's t-test for the maternal and cord group, pooled Student's t-test for the HIV-1 seronegative blood donor and HIV-1 seropositive groups, and two-tail Fisher's Exact test for the isotypic response to gp41 and p24.

5.0 RESULTS

5.1 C-reactive protein estimation in healthy individuals

Out of the 40 maternal sera, 10 were found to have C-reactive protein>6mg/l. These samples also had high mean level of IgM (6.7g/l). These findings were considered to be consistent with the presence of infection. Therefore these sera together with their corresponding cord sera were excluded from the study. Of the HIV-1 seronegative sera, only 4 were positive for this test. However, the concentration of IgM in each of these sera was within the range of the mean level for HIV-1 seronegative sera and therefore were included in the study.

5.2 Immunoglobulin levels in pregnant mothers and their newborns

Table 5. Mean levels of immunoglobulin classes in maternal and cord sera

Class	Maternal (n=30)	Cord (n=30)	p value
IgG(g/l)	19.9 (5.16)*	16.82 (3.75)	p=0.5
IgA(g/l)	2.79 (0.84)	<0.31	p=0
IgM(g/l)	3.08 (1.71)	<0.25	p=0
IgD(IU/ml)	68.33 (57.3)	0.00	p=0
IgE(IU/ml)	2689.33 (2883.09)	0.00	p=0

* Values in brackets indicate standard deviations.

Immunoglobulin levels in maternal and the corresponding cord sera are shown in Table 5. The mean IgG level in maternal sera was slightly higher than that in cord sera, however this difference was not statistically significant. Twenty percent of the cord sera had IgG

levels higher than the mean maternal IgG concentration. The other immunoglobulin isotypes (IgA, IgM, IgD and IgE) were not detected in cord sera. IgD and IgE levels were undetectable by the assay in 6% and 10% of the maternal sera respectively.

Table 6. Mean levels of IgG subclasses in maternal and cord sera

Subclass	Maternal (n=30)	Cord (n=30)	p value
IgG1(g/l)	11.99 (5.4)*	12.77 (5.63)	p=0.49
IgG2(g/l)	3.74 (2.92)	0.86 (1.05)	p<0.0001
IgG3(g/l)	2.38 (2.03)	2.11 (2.38)	p=0.67
IgG4(g/l)	1.11 (1.01)	0.68 (1.2)	p=0.07

* Values in brackets indicate standard deviations

The IgG subclass profiles in maternal and cord sera are presented in Table 6. The mean IgG1 level in cord sera was higher than that of maternal sera but this was not statistically significant. However, 50% of the cord sera had IgG1 concentrations higher than the mean IgG1 level for maternal sera. IgG2 levels were significantly higher in maternal than in the corresponding cord sera. There was no significant difference in the mean maternal and cord IgG3 levels. The higher level of IgG4 in maternal sera compared to cord sera was of a borderline statistical significance. Twenty three percent of the cord sera had IgG4 levels higher than the mean IgG4 level for maternal sera.

Table 7.Fetal/maternal total IgG and IgG subclass ratios at term

	Unmatched ratios	Matched ratios
IgG	0.85	0.89
IgG1	1.07	1.72
IgG2	0.23	0.34
IgG3	0.89	1.20
IgG4	0.61	1.53

The fetal/maternal ratios of the mean fetal and maternal IgG and IgG subclass levels at full term are shown in Table 7. The matched ratios of all subclasses are more than 1 except for IgG2. There was a wide variation in the individual IgG4 values in both maternal and cord sera. This explains the discrepancy between the unmatched and matched fetal/maternal ratios for IgG4.

5.3 Immunoglobulin levels in HIV-1 seronegative blood donors and in HIV-1 seropositive individuals

Table 8. Mean serum levels of immunoglobulin classes in HIV-1 seronegative controls and in HIV-1 seropositive subjects

Class	HIV-1-ve (n=50)	HIV-1+ve (n=50)	p value
IgG (g/l)	17.97 (4.3)*	30.27 (9.75)	p=0.0001
IgA (g/l)	2.07 (0.88)	3.38 (2.05)	p=0.0001
IgM (g/l)	1.80 (1.22)	3.54 (2.39)	p<0.0001
IgD (IU/ml)	52.26 (34.9)	112.9 (67.33)	p=0.0001
IgE (IU/ml)	2965 (2192)	3446.4 (2663.43)	p=0.326

* Values in brackets indicate standard deviations.

The mean serum levels of all immunoglobulin classes, as seen in Table 8, were found to be higher in HIV-1 seropositive sera than in sera from apparently healthy HIV-1 seronegative controls. In the seropositive group, individuals with immunoglobulin concentrations higher than the mean control levels were 88%, 68%, 68%, 76%, and 46% of the total for IgG, IgA, IgM, IgD, and IgE respectively. A statistically significant increase in IgG, IgA, IgM, and IgD levels was evident. The mean level of IgE in the HIV-1 seropositive group was also raised but the increase was not statistically significant when compared to the control group. IgD levels were undetectable by the assay in 10% of the control sera and in 2% of the HIV-1 seropositive sera. IgE levels were undetectable in 12% of the control sera and in 6% of the HIV-1 seropositive sera.

Table 9. Mean serum levels of immunoglobulin classes in asymptomatic HIV-1 seropositive individuals and in AIDS patients

Class	Asymptomatic HIV-1+ve (n=30)	AIDS patients (n=20)	p value
IgG(g/l)	28.35 (8.5)*	33.12 (11.02)	p=0.092
IgA(g/l)	2.56 (1.5)	4.55 (2.58)	p=0.0006
IgM(g/l)	2.96 (2.1)	4.41 (2.58)	p=0.033
IgD(IU/ml)	83.2 (56.27)	157.45 (58.11)	p=0.0001
IgE(IU/ml)	3794.67 (2945.6)	2924 (2138)	p=0.262

* Values in brackets indicate standard deviations.

Analysis of the immunoglobulin concentrations in the two phases of HIV-1 infection revealed an increasing trend for IgG, IgA, IgM and IgD with evolution of the disease from asymptomatic phase to AIDS phase (Table

9). This trend was particularly marked for IgD, whereby the level almost doubled as patients evolved from asymptomatic phase to AIDS. In the asymptomatic group, those with immunoglobulin concentrations higher than the mean levels in the controls were 83.3% for IgG, 60% for IgA, 56% for IgM, 60% for IgD and 50% for IgE. In the AIDS phase, individuals with immunoglobulin levels higher than the mean control levels were 95% for IgG, 75% for IgA, 75% for IgM, 100% for IgD, and 40% for IgE. With disease progression to AIDS, IgE levels decreased to a level almost identical to that of seronegative controls. The IgE elevation seen in seropositives (Table 8) is therefore largely due to the high levels in the asymptomatic HIV-1 seropositive group.

Table 10. Mean levels of IgG subclasses in HIV-1 seronegative blood donors and in HIV-1 seropositive subjects

Subclass	HIV-1-ve (n=50)	HIV-1+ve (n=50)	p value
IgG1(g/l)	12.76 (4.39)*	20.95 (8.56)	p=0.0001
IgG2(g/l)	1.76 (1.16)	1.55 (1.78)	p=0.475
IgG3(g/l)	1.5 (0.97)	5.51 (4.63)	p<0.0001
IgG4(g/l)	0.48 (0.53)	0.85 (1.92)	p=0.078

* Values in brackets indicate standard deviations.

In HIV-1 seropositive subjects, the mean IgG subclass levels obtained showed a statistically significant increase in IgG1 and IgG3 levels compared to the HIV-1 seronegative controls (Table 10). In the seropositive group, subjects with IgG subclass levels higher than the mean control levels were 84%, 28%, 88%, and 38% for IgG1, IgG2, IgG3,

and IgG4 respectively. The IgG4 concentration in the HIV-1 seropositive group was higher than that of the control group but this difference was not statistically significant.

Table 11. Mean levels of serum IgG subclasses at the two stages of HIV-1 infection

Subclass	Asymptomatic HIV-1+ve(n=30)	AIDS patients (n=20)	p value
IgG1(g/l)	21.31 (7.96)*	20.42 (9.57)	p=0.722
IgG2(g/l)	1.78 (1.85)	1.21 (1.65)	p=0.269
IgG3(g/l)	4.29 (3.3)	7.34 (5.7)	p=0.022
IgG4(g/l)	1.16 (2.4)	0.38 (0.54)	p=0.0009

* Values in brackets indicate standard deviations.

Analysis of the mean IgG subclass levels was done in the two phases of HIV-1 infection, (Table 11). The mean IgG1 levels remained almost constant in both phases. IgG2 levels slightly decreased with disease progression to AIDS but this change was not statistically significant. IgG3 levels doubled with evolution from asymptomatic to AIDS phase, a statistically significant increase. IgG4 levels were significantly higher in asymptomatic HIV-1 seropositives when compared to seronegative controls (p=0.0001), and decreased dramatically in the AIDS phase, to reach a level almost identical to that of the control group.

5.4 Anti-HIV-1 IgG subclass response to gp41 and p24 HIV-1 antigens

Table 12. Number of sera with a positive IgG subclass reactivity to gp41

Subclass	Asymptomatic phase (n=11)	AIDS phase (n=11)	p value
IgG1	10	11	p=1.0
IgG2	4	6	p=0.67
IgG3	4	1	p=0.31
IgG4	6	0	p=0.012

All sera showed some reactivity to gp41 as shown in Table 13. In one serum from the asymptomatic group, reactivity to gp41 was only limited to IgG3. Of the sera from asymptomatic subjects 4 (36.4%) showed reactivity in all subclasses. One of the sera from AIDS patients showed reactivity in all IgG subclasses except for IgG4.

Table 13. Number of sera with a positive IgG subclass reactivity to p24

Subclass	Asymptomatic phase (n=11)	AIDS phase (n=11)	p value
IgG1	6	8	p=0.66
IgG2	2	0	p=0.48
IgG3	7	0	p=0.004
IgG4	4	6	p=0.67

All sera from asymptomatic individuals showed reactivity in more than one subclass (Table 13). Response was only limited to IgG1 and IgG4 subclasses in the AIDS cases.

6.0 DISCUSSION

Previous studies on human serum immunoglobulin levels in Tanzania have mainly dealt with quantitation of IgG,IgA and IgM isotypes (33,34,35). In Tanzania, there is no documented study on IgG subclass levels. Serum levels of IgD have also not been reported. IgE levels in healthy subjects are also not documented. The present study has analyzed serum levels of the five immunoglobulin isotypes as well as those of IgG subclasses in full term pregnant mothers and their newborns, apparently healthy HIV-1 seronegative blood donors and HIV-1 seropositive individuals. In addition, anti-HIV-1 IgG subclass response to gp41 and p24 has been studied. Isotypic response to HIV-1 antigens has been reported in few studies, none of which have been done in Tanzania.

The various techniques used for immunoglobulin quantitation in this study are well standardized and widely used. The IgD and IgE standards used in the single radial immunodiffusion technique gave consistent results in all plates, suggesting that there was no significant assay variation between the plates. Results obtained for mean serum immunoglobulin levels are therefore likely to be reproducible. The SRID plates used in IgD and IgE quantitation failed to detect levels of these two immunoglobulin isotypes in some of the sera. This could be partly due to failure of the technique to detect very low levels, or may be the individuals did not have these isotypes in their sera. Absence of IgD in serum is known to occur in some individuals, and this phenomenon is genetically determined (166). Rowe and co-worker's also reported absence of detectable IgD levels in 10%

of Gambian adults (31). The IgG subclass ELISA technique used was reported by the manufacturer to be highly sensitive, detecting as low as 3.3 ug/l, 17 ug/l, 3.4 ug/l, and 12 ug/l of IgG1, IgG2, IgG3 and IgG4 respectively. The standard curves obtained for both the commercially pre-coated plates and those coated by the author gave correct values for the kit controls as well as for the WHO human IgG subclass control. Quantitation of total IgG concentration by nephelometer yielded higher values than those obtained by summation of the individual mean IgG subclass levels quantified by ELISA. This discrepancy is due to the differences in the two assay techniques. However, there was a strong positive correlation between these two sets of values (correlation coefficient=0.8) suggesting comparability between the two assay methods. Inspite of this observation, the IgG values obtained by nephelometer were considered to be more accurate than the calculated IgG concentration based on four separate IgG subclass assays, and were therefore used for comparative analysis between the various study groups. The monoclonal antibodies used in this study have been evaluated for their specificities for human IgG subclasses in a WHO-collaborative study involving several laboratories (96). Investigators in this study reported low reactivity for IgG2 monoclonal antibody, an observation that was also noted in the present study. However, this observation does not influence the results obtained. The compact structure of IgG2 subclass together with the limited number of IgG2 specific monoclonal antibodies are among the reasons why IgG2 is generally considered to be the most difficult IgG subclass to detect.

The mean levels of IgG, IgA and IgM in maternal, and HIV-1 seronegative sera, and the IgG level in cord sera found in this study are comparable to those previously reported by other investigators in Tanzania (33,35), who used single radial immunodiffusion technique. These could therefore be considered as reference values for Tanzanian adults. The mean level for IgM in maternal sera was significantly higher ($p=0.001$) than that found in HIV-1 seronegative male blood donors. Rowe and co-workers (31) previously reported higher mean IgM concentrations in non-pregnant females than males in Gambia, and postulated that perhaps the sex difference is due to the balance of male and female sex hormones acting as a factor in the control of IgM metabolism. If proved, this hypothesis could also explain the difference observed in this study. However, it will be important to examine non-pregnant women matched for age to further elucidate on this. The mean IgD and IgE values in the maternal sera were remarkably higher than those reported in other non-tropical series (167). Litwin and co-workers have previously demonstrated an association between certain Gm genetic markers and serum IgD levels in Caucasians (166). Whether this observation explains the high IgD levels found in pregnant mothers in the present study remains to be established. It is also possible that the high IgD levels seen in pregnant mothers in this study is due to the heightened antigenic challenge in pregnancy. The high mean IgE levels may be due to a co-existing infection of the subjects with helminthic parasites. Although this aspect was not analyzed in the present study, high serum IgE levels in individuals with helminthiasis have been reported in a previous study conducted in

a tropical environment (32). The standard deviations for the mean levels of IgD and IgE were strikingly high. This is explained by the fact that the distribution of the serum levels of these isotypes in human populations does not conform to the normal distribution pattern. For example, IgD distribution has been shown to be bimodal or even trimodal (168). Absence of IgA, IgM, IgD and IgE in cord sera as seen in this study signifies lack of placental transfer for these isotypes, a finding which is in agreement with documented reports on placental transfer of human immunoglobulins (16,17,22). It also indicates that the neonates had no intrauterine infections.

The serum IgG subclass concentrations found in the maternal and cord sera clearly indicate that all four IgG subclasses cross the placenta, a finding which is consistent with reported data by other investigators (41,42,44,47,48). In this study the matched fetal/maternal ratio for IgG2 subclass at term was 0.34, meaning that the mean cord IgG2 concentration was less than the concentration in the corresponding maternal sera. The opposite was the case for the other subclasses, whose matched ratios were more than 1. This finding compares very well with reported data on the transfer of IgG subclasses across the placenta (47,169), in which it has also been shown that transfer of IgG2 subclass is less efficient than that of the other subclasses. Active transport of immunoglobulins across the placenta has been postulated before (22). The finding of higher levels of IgG in 20% of the cord sera in this study adds further evidence for the possible existence of an active placental transfer of the IgG subclasses. The higher mean IgG2 level in maternal sera as compared to the level in

cord sera also partly explains the relatively higher mean total IgG level in the maternal sera.

Placental transfer of the human IgG subclasses has been hypothesised to take place through an Fc receptor-mediated mechanism (49), and that *in situ* Fc receptor binding affinity of the four subclasses to human placental tissues has been reported to be in the order IgG1=IgG3>IgG4>IgG2 (51). A similar order of IgG subclass binding affinity was demonstrated in a study of Fc receptors in placental homogenates (48). The unmatched fetal/maternal ratios obtained in this study follow the same order of magnitude. This finding, therefore, further supports the hypothesis of Fc receptor-mediated placental transfer for the human IgG subclasses. Reported studies on Fc receptors in the human placenta were largely done in Caucasians. The finding of a similar transfer priority of IgG subclasses in a tropical African population suggests that probably there are no racial or geographical differences in the placental transfer of human immunoglobulins. The reported order of binding affinity, and subsequent preferential transfer of the IgG subclasses in that order could be due to the differences in the length and flexibility of the hinge region among the subclasses. The IgG1 hinge is freely flexible while IgG2 hinge flexibility is restricted by its shortness and lack of glycine residues (42). IgG3 is known to have an extended and highly flexible hinge region, whereas the flexibility of IgG4 is thought to be intermediate between that of IgG1 and IgG2 (42). It seems, therefore, that the descending order of hinge flexibility for the IgG subclasses is IgG3>IgG1>IgG4>IgG2, which resembles the order of binding affinity of

IgG subclasses to Fc receptors on the human placenta. Hinge flexibility influences effector functions of the IgG subclasses such as Fc receptor-binding (42,54). Therefore preferential transfer of the subclasses across the placenta is likely to be determined by the flexibility of the hinge region of the respective IgG subclass.

Another objective of this study was to analyze the serum levels of immunoglobulin isotypes in HIV-1 seronegative sera and compare them with those found in HIV-1 seropositive sera, with a view to elucidating any characteristic pattern of serum immunoglobulin concentrations in HIV-1 seropositive individuals. The findings obtained revealed significantly elevated mean serum levels of all immunoglobulin classes (except IgE) in HIV-1 seropositive sera when compared to the mean levels in age and sex-matched seronegative controls. Similar findings have been reported in previous studies which demonstrated hypergammaglobulinemia in HIV-1 infection and AIDS in terms of elevated serum IgG and IgA (143), IgM (143), IgD (149), and more recently, IgE (167). This pattern of hypergammaglobulinemia in HIV-1 infection is distinct from patterns observed in other infectious diseases. For example, in Epstein-Barr virus mononucleosis, IgM and IgE isotypes are most strikingly elevated in the early weeks of the disease, and IgD is generally the least elevated (170). In tuberculosis, IgM levels are often high, but IgD is present at normal or even subnormal levels (171). In *Mycoplasma* and bacterial pneumonias, immunoglobulins are rarely elevated above normal (172). In other immunodeficiency disorders such as in Hodgkin's disease, IgD is not elevated even in the presence of similar opportunistic infections as those found in AIDS (173).

Therefore, the polyisotypic hypergammaglobulinemia in HIV-1 infection as seen in the present study as well as in others (131,142,143), seems to be a unique immunological phenomenon. Other investigators who have reported similar findings have attributed this phenomenon to a polyclonal activation of B lymphocytes (142,143). Presence of other known polyclonal B cell activators such as Epstein-Barr virus and CMV was not investigated for in this study. However, on the basis of other reports which have convincingly demonstrated the existence of polyisotypic hypergammaglobulinemia in HIV-1 infected individuals even in the absence of co-existing infection with other polyclonal B cell activating agents, the hypergammaglobulinemia seen in HIV-1 seropositive sera in the present study is suggested to be a manifestation of polyclonal B cell activation.

In the present study, the average increases in the mean levels of immunoglobulin classes in the HIV-1 seropositive group relative to the mean levels in the seronegative control group are 1.6-fold for IgG and IgA, 2-fold for IgM and 2.2-fold for IgD (ie.IgD>IgM>IgG=IgA). Mizuma and co-workers (149) reported similar magnitudes of increase for IgG, IgA and IgM and a much higher one for IgD in a study involving HIV-1-infected homosexual subjects, intravenous drug abusers and hemophiliacs. The order of arrangement of immunoglobulin heavy chain genes on the chromosome is reported to be IgM->IgD ->IgG3->IgG1->IgE2->IgA1->IgG2->IgG4->IgE1->IgA2 (4). Isotypic switching during genetic expression of immunoglobulin heavy chains usually follows a similar order. Lack or defect in isotypic switching due to T4 cell depression in HIV-1 infection could explain the order of increase in the

immunoglobulin classes in the HIV-1 seropositive group as seen in this study.

The levels of all immunoglobulin isotypes except for IgE remained elevated in both the asymptomatic and AIDS phases, suggesting that abnormal B cell activation is characteristic of patients with mild as well as severe manifestations of HIV-1 infection. Other investigators have reported similar findings (143,149). The finding of significantly elevated mean IgA and IgM levels in AIDS patients as seen in this study has also previously been reported (143,174). Some reports have attributed this to the significantly elevated IgA and IgM rheumatoid factors seen in AIDS patients when compared to asymptomatic HIV-1 seropositive subjects (175), and others have claimed this to be due to the presence of opportunistic infections in AIDS patients (143). However, in the present study no attempt was made to look for rheumatoid factors in the sera.

The persistent increase in the mean IgD levels in the AIDS phase as seen in this study is in contrast to the findings reported by other investigators. For example, in the study by Mizuma and co-workers (149), a decline in the mean IgD level was seen in AIDS phase, although it remained high when compared to the mean level in HIV-1 seronegative controls. IgD production has been shown to be a marker of B cell immaturity (176). Whereas increased production of this isotype occurs in AIDS patients with opportunistic infections, increased production is often absent in patients with AIDS-associated Kaposi's sarcoma alone (177). The finding of high mean IgD concentration in AIDS sera in the present study is therefore partly explained by the fact that all AIDS

patients studied had opportunistic infections and none had Kaposi's sarcoma. Some of the patients in Mizuma's study (149) had Kaposi's sarcoma. It has also been observed previously that many bacterial species that are common in the upper respiratory tract have Fc receptors for IgD and may thus exert mitogenic effects on IgD - expressing lymphocytes (178). If some of these receptors are similar to IgD-binding factor (IgD-BF), then a possible explanation for the increased IgD levels in patients with AIDS could be that the recurrent upper respiratory infections often seen in AIDS patients are associated with expression of IgD-BF which then activates IgD production. This postulate deserves further investigation, especially in view of the reported findings of *in vitro* formation of immunoglobulin binding factors such as IgE-BF by T cells from patients infected with HIV-1 (179). In addition, lack of downregulation due to lack of T4 cell could also lead to accumulation of IgD. Previous studies have reported elevation of IgE levels in HIV-1-infected individuals (167). Increased occurrence of IgE-BF has also been reported in association with HIV-1 infection (179), and this immunoglobulin binding factor favours increased production of IgE. It is therefore rather surprising that the mean levels of IgE found in the HIV-1 seropositive group and in seronegative controls in the present study do not differ significantly. These levels, however, are markedly higher than those reported in other non-tropical series (167), and could possibly be due to a co-existing helminthiasis. Any significant IgE increase due to HIV-1 related polyclonal B cell activation would therefore be masked by the increase attributable to parasitic activation. In the present study, 50% of the

asymptomatic HIV-1 seropositive individuals had IgE levels which were higher than the mean concentration for the controls. Demonstration of a true increase in IgE levels as a result of polyclonal activation of B lymphocytes would therefore require exclusion of infection with helminthic parasites in the study groups.

The mean serum levels of all IgG subclasses in the seronegative controls were higher than those reported in Caucasians (54,58). IgG levels in people living in the tropics have been reported to be higher than those reported in residents of temperate regions (26,27,28,31). This was also observed in the present study, and partly explains the higher mean IgG subclass concentrations in the study subjects. Since the serum levels of IgG subclasses have been shown to be partly influenced by some genetic factors (56), it may be that the higher levels of IgG subclasses found in this study partly have a genetic basis. However, this remains to be established. The mean serum IgG subclass levels found in this study display a characteristic IgG1 and IgG3 predominance as reported in studies involving homosexually and parenterally HIV-1-infected individuals (76,77,151). The insignificant change in mean IgG2 levels, with a tendency to decrease in HIV-1 seropositive individuals as seen in the present study has also been reported elsewhere (77). What brings about these serum IgG subclass changes seen in association with HIV-1 infection is not yet established. However, in other viral infections an IgG1 and IgG3 subclass antibody restriction has been widely documented (67,68,69,70,71,72), and attributed to the viral protein antigens. There is no reason to dispute the existence of a similar IgG subclass

restriction in HIV-1 infection. The mean IgG3 subclass level in AIDS sera was almost two times higher than that in sera from asymptomatic blood donors. A similar observation was noted for the mean IgD levels in the same groups. In view of the close proximity of the genes for the heavy chains of IgD and IgG3 on the chromosome, it is tempting to postulate that a defective regulation of the downstream switch in the heavy chain locus may give rise to a prominent generation of both IgD and IgG3 isotypes in AIDS patients. The downstream switching mechanism in the expression of heavy chain genes has been reported to be influenced by some isotype-specific switch factors such as IL-4 and IL-5 (42). The role of such factors in influencing the production of immunoglobulins in patients with AIDS remains to be established. Another striking finding is the significantly elevated mean IgG4 level in asymptomatic subjects when compared to HIV-1 seronegative controls. This is in contrast to reported data from other studies (77), in which IgG4 levels have been shown to be normal in HIV-1 seropositive individuals irrespective of the phase of the disease. Since repeated antigenic challenge is known to give rise to an IgG4-restricted response (87,88), the elevated IgG4 subclass level seen in the asymptomatic group could be due to repeated stimulation by HIV-1 antigens. However, the reason why the IgG4 level should fall back to lower level in the AIDS phase is less clear.

The other aim of the present study was to establish the pattern of IgG subclass response to some of the envelope and core antigens of HIV-1. This study has demonstrated an anti-HIV-1 IgG subclass response to gp41 in all four subclasses, mainly IgG1 and IgG4 in the

asymptomatic phase and IgG1 and IgG2 in the AIDS phase. Predominance of IgG1 subclass response was found in both phases of the disease, and this response declined with evolution to AIDS. Absence of IgG4 response to gp41 was a remarkable feature. Chiodi and co-workers reported an IgG1 and IgG2 restricted response to overlapping peptides derived from the gp41 transmembrane protein of HIV (152). It has previously been shown that IgG subclass restriction is determined by the nature of the antigen: IgG1 and IgG3 responses are restricted to protein antigens (42,44,54,60) while IgG2 restriction is characteristic for polysaccharide antigens (61,62,63). Presence of both protein and polysaccharide components in the gp41 antigen therefore explains the finding of IgG1, IgG2 and IgG3 responses to this antigen. The predominance of IgG1 response is rather interesting. The mechanisms determining the distribution of IgG subclasses are poorly understood but evidence favours the participation of T-helper cells in the IgG1 isotypic restriction observed in some autoimmune responses (180). Also in a murine model of chronic infection with the cestode parasite *Mesocestoides corti*, the isotypic restriction to IgG1 has been shown to be the direct consequence of a negative regulatory effect on B cells, of a clonal subpopulation of parasite-specific CD4+ T-helper cells (181). These data suggest that suppressor mechanisms might account for the IgG1 restriction to gp41. The finding of IgG3 subclass response to gp41 is rather striking, and was never reported in a previous study on isotypic restriction to HIV-1 antigens(74). IgG3 antibody to viral envelope proteins has been shown to have a potent neutralizing effect (150). This finding in the present study is

therefore of interest because of its applied value in relation to vaccine development. However a study involving a larger sample size is recommended in order to ascertain this finding. Loss of IgG3 and IgG4 reactivity to HIV-1 has been shown to occur with progression of the disease (151). This trend was also observed in the present study. Overwhelming antigenemia is reported to occur in the AIDS phase (128,131,137). Such a situation could lead to the clearance of specific IgG subclasses through formation of soluble immune complexes, leading to loss of reactivity to HIV-1 antigens.

Response to p24 was also prominent in the IgG1 subclass in both phases of the disease. In the asymptomatic phase, a typical IgG1 and IgG3 subclass response to a protein antigen was noted, whereas in the AIDS phase the response was IgG1 and IgG4 restricted. Presence of an IgG4 response to p24 appears to be a bad prognostic sign. While IgG1 and IgG3 responses to **gag** proteins have previously been demonstrated in asymptomatic HIV-infected individuals (74), IgG4 response to **gag** proteins has been elicited mainly in haemophiliacs (74). The IgG4 response in hemophiliacs has been attributed to repeated antigenic stimulation associated with repeated transfusions of clotting factor products. It would be of interest to establish whether a similar mechanism of repeated antigenic challenge takes place in heterosexually transmitted HIV-1 infection. Surprisingly, the antigen-specific IgG4 response in both phases does not parallel the mean IgG4 subclass levels in the asymptomatic and AIDS phases. This could be due to the small sample size studied for IgG subclass response to HIV-1 antigens.

7.0 CONCLUSION

In conclusion, several findings obtained from this study do support data reported in literature. Analysis of maternal and cord sera has demonstrated placental transfer of all IgG subclasses in an order that resembles the reported order of the binding affinities of the IgG subclasses to the Fc receptors in the human placenta. This interesting observation lends support to the hypothesis of Fc receptor-mediated mechanism for placental transfer of the human IgG subclasses, and perhaps racial and geographical factors do not influence the mechanism of transfer of immunoglobulins across the human placenta.

Polyisotypic hypergammaglobulinemia and serum IgG subclass dysbalances have been demonstrated in HIV-1 seropositive individuals living in a region where heterosexual HIV-1 transmission is reckoned to dominate (104,105). Similarity of these findings to those reported in individuals infected through homosexual practices and parenteral route, suggests that these phenomena are probably not influenced by the mode of HIV-1 transmission. A similar conclusion can be drawn for the IgG subclass response to gp41 and p24. However, the findings obtained in the analysis of anti-HIV-1 IgG subclass response to gp41 and p24 should be considered with caution in view of the small sample studied.

Some of the knowledge obtained from this study is of clinical significance. The finding of lack of placental transfer for IgA, IgM, IgD and IgE is of diagnostic value in that presence of these isotypes in a newborn can be taken as a strong evidence for intrauterine infection. Some tests based on detection of antigen-specific IgM are already in use, as for example in the diagnosis of congenital

toxoplasmosis and rubella. Elevation of serum immunoglobulins as seen in the HIV-1 seropositive group could be used as a marker for HIV-1 infection, and demonstration of serum IgG subclass dysbalances could be used for monitoring disease progression. The isotypic response pattern to gp41 and p24 found in this study could be useful in HIV-1 diagnosis, monitoring of disease progression and in the development of candidate vaccines.

8.0 RECOMMENDATIONS

In view of the findings obtained in this study, the following recommendations are pertinent.

1. Further studies on immunoglobulin levels in non-pregnant women.

2. Studies on the association between genetic factors and serum levels of immunoglobulins in Tanzanian subjects.

3. A study on IgE levels in HIV-1 seropositive and seronegative subjects in which helminthic infection has been excluded.

4. Studies on the prevalence of IgA and IgM rheumatoid factors and various immunoglobulin binding factors in HIV-1-infected subjects.

5. A study on the IgG subclass levels in the different phases of HIV-1 infection in HIV-1 seropositive children.

6. A study on IgG subclass response to gp41 and p24, involving a larger sample size.

9.0 REFERENCES

1. Roitt I: Essential Immunology. Sixth Ed. Oxford. Blackwell Scientific publications 1988; 31-54.

2. Gottlieb M, Schanker H M, Fan P T, Saxon A, Weisman J D: Pneumocystis pneumonia-Los Angeles. MMWR 1981; **30** :250-252.

3. WHO/SPA/GEN/ 87.1, March 1987.

4. Roitt I, Brostoff J, Male D: Immunology, Gower Medical Publishing Ltd, London, England, 1985.

5. Nomenclature for human immunoglobulins. Bull WHO 1964; **30**:447-450.

6. Nisonoff A, Hopper J E, Spring S B: The antibody molecule. Academic Press Inc New York, 1975; 264.

7. Benacerraf B, Unanue E R: Textbook of Immunology, Williams & Wilkins, Baltimore, 1979.

8. Porter R R: Hydrolysis of rabbit Y-globulins and antibodies with crystalline papain. Biochem J. 1959; **73**: 119.

9. Cohen S, Milstein C: Structure of antibody molecules. Nature. 1967; **214**:449-452.

1967; **214**:449-452.

10. Nisonoff A, Wissler F C, Woernley D L. Properties of rabbit antibody isolated by specific adsorption. Arch Bioch Biophys. 1960; **88**:241-249.

11. Hershgold E J, Cordoba P, Charache P, Gitlin D: A crystalline fragment from human gamma-globulin. Nature. 1963; **199**:284-285.

12. Tonegawa S: Sequence of a mouse germline gene for a variable region of an immunoglobulin light chain. Proc Natl Acad Sci USA. 1978; **75**:1486.

13. Heidelberger M, Pedersen K O, Tiselius A: Ultracentrifugal and electrophoretic studies on antibodies. Nature. 1936; **138**:165

14. Wu T T, Kabat E A: An analysis of the sequences of the variable regions of Bence-Jones proteins and myeloma light chains and their implications for antibody complementarity. J Exp Med. 1970; **132**: 211

15. Feinstein A, Richardson N, Taussig M J: Immunoglobulin flexibility in complement activation. Immunol Today. 1986; **7**: 169.

16. Gitlin D, Kumate J, Urrusti J, Morales C: The selectivity of the

human placenta in the transfer of plasma proteins from mother to fetus. J Clin Invest. 1964; 73(10): 1938-1951.

17. Connon A F: Immunoglobulin levels for maternal and fetal serum, amniotic fluid and fetal gastric juice in normal pregnancy. Aust J Exp Biol Med Sci. 1970; **48**: 691.

18. Burton D R, Gregory L, Jefferis R: Aspects of the molecular structure of the IgG subclasses. Monograph Allergy. 1986; **19**: 7.

19. Calvanico N J: Immunochemistry of Proteins. Plenum, New York.1979; 1-85.

20. Bandilla K K, McDuffie F C, Gleich G J: Immunoglobulin classes of antibodies produced in the primary and secondary response in man. Cl Exp Immunol. 1969; 5: 627-641.

21. Peacock D B, Jones J V, Gough M: The immune response to OX 174 in man: Primary and Secondary antibody production in normal adults. Cl Exp Immunol. 1973; 13: 497-513.

22. Kohler P F, Farr R S: Elevation of cord over maternal IgG immunoglobulin. Evidence for an active placental IgG transport. Nature. 1966; **210**: 1070-1071.

23. Ishizaka K, Ishizaka T, Hornbrook M M: Physicochemical

properties of human reaginic antibody. IV.Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol. 1966; **97**: 75-85.

24. Bennich H H, Ishizaka k, Jahansson S G O, Rowe D S, Stannworth D R, Terry W O: Immunoglobulin E, a new class of human immunoglobulin. Bull WHO. 1968; **38**:151.

25. Buckley C E, Dorsey F C: The effect of aging on human serum immunoglobulin concentrations. J Immunol. 1970; **105**: 964-972.

26. Milan D G: Plasma protein levels in normal individuals. J Lab Clin Med. 1946; **31**: 285-290.

27. Stainer M W, Thompson M D: The serum protein levels of newborn African infants. Archs Dis Childn. 1954; **29**: 110-112.

28. Edozien J C: The serum proteins of healthy adult Nigerians. J Clin Path. 1957; **10**: 276-279.

29. Edozien J C: The development of the serum protein pattern in Africans. J Clin Path. 1961; **14**: 644-653.

30. Oyeyinka G O, Salimonu L S *et al*: Range of normal serum immunoglobulin (IgG, IgA, IgM) values in Nigerians. Afr J Med Sci. 1984; **13**(3/4): 169-176.

31. Rowe D S, McGregor I A, Smith S J, Hall P, Williams K: Plasma immunoglobulin concentrations in a West African (Gambian) community and in a group of healthy British adults . Clin Exp Immunol. 1968; **3**: 63-79.

32. Johansson S G O, Mellbin T, Vahlquist B: Immunoglobulin levels in Ethiopian pre-school children with special reference to high concentrations of immunoglobulin E. Lancet. 1968; **i**: 1119-1121.

33. Nantulya V M, Lindqvist K J: Serum protein concentrations of normal Tanzanian subjects. E Afr Med J. 1973; **50**: 576-580.

34. Voller A, Lelijveld J, Matola Y G: Immunoglobulin and malaria indices at different altitudes in Tanzania. J Trop Med Hyg. 1971; **74**(145):45-52.

35. Shao J F, Matre R: Serum immunoglobulin levels in various age groups of normal Dar es Salaam Tanzanian population (in press).

36. Drays S: Three g-globulins in normal serum revealed by monkey precipitations. Science. 1960; **133**: 1313-1314.

37. Grey H M, Kunkel H G: H-chain subgroups of myeloma proteins and normal 7s g-globulin. J Exp Med. 1964; **120**: 253-266.

38. Terry W D, Fahey J L: Subclasses of human g-globulin based on

differences in heavy polypeptide chains. Science. 1964; **146:** 400-401.

39. Kunkel H G, Prendergast R A: Subgroups of gamma A immune globulin. Proc Soc Exp Biol Med. 1963; **122:** 910-913.

40. Vaerman J P, Heremans J F: Subclasses of human immunoglobulin A based on differences in the alpha polypeptide chains. Science. 1966; **153:** 647-649.

41. Schur P H: IgG subclasses-a review. Annals of Allergy. 1987; **58:**89-99.

42. Vlug A, Van Remortel P: The structure and function of human IgG subclasses. European J Clin Lab. Dec.1989; 26-35.

43. Pumphrey R: Computer models of the human immunoglobulins: Shape and segmental flexibility. Immunol Today. 1986; 7:174-178.

44. Shakib F, Stanworth D R: Human IgG subclasses in health and disease. Part I Ric Clin Lab. **10:** 463-479 Part II Ric Clin Lab. 1980; **10:** 561-580.

45. Bernier G M, Ballieux R E, Tominaga K T, Putnam F W: Heavy chain subclasses of human gamma G. globulin. J Exp Med. 1967; **125:** 303.

46. Wang A C, Faulk W P, Stuckey M A, Fudenberg H H: Chemical differences of adult, fetal and hypogammaglobulinemic IgG immunoglobulins. Immunochemistry. 1970; **7**: 703-708.

47. Virella G, DePascale A, Lippi U: Placental transfer of a human IgG2 monoclonal protein. Experimentia. 1971; **27**: 324-325.

48. McNabb T, Koh T Y, Dorrington K J, Painter R H: Structure and function of immunoglobulin G and fragments to placental membrane preparations. J Immunol.1976; **177**:882-888.

49. Brambell F W R: The transmission of immunity from mother to young and the catabolism of immunoglobulins. Lancet. 1966; **2**:1087-1093.

50. Matre R, Tonder O, Endersen C: Fc receptors in human placenta. Scand J Immunol. 1975; **4**:741-745.

51. Matre R: Similarities of Fc receptors on trophoblasts and placental endothelial cells. Scand J Immunol. 1977; **6**:953-958.

52. French M A H, Harrison G: Serum IgG subclass concentrations in healthy adults: A study using monoclonal antisera. Clin Exp Immunol. 1984; **56**: 473-475.

53. Lee S I, Heiner D C, Wara D: Development of serum IgG subclass

levels in children. Monogr Allergy. 1986; **19**: 108-121.

54. Normansell D E: Human immunoglobulin subclasses: Review. Diag Immunol. 1987; **5**:115-128.

55. Radl J, Sepers J M, Skvaril F, Morell A, Hijmans W: Immunoglobulin patterns in humans over 95 years of age. Clin Exp Immunol. 1975; **22**:84.

56. Morell A, Skvaril F, Steinberg A G, Van Loghem E, Terry W D: Correlation between concentrations of the four subclasses of IgG and Gm allotype in normal human sera. J Immunol. 1972; **108**:195-206.

57. Apampa O O, Salimonu L S, Williams A I O, Osunkoya B O: Serum levels of immunoglobulin G subclasses in Nigerians. Trop Geogr Med. 1980; **32**:50-52.

58. Djurup R, Sondergaard I, Magnusson C G M *et al*: IgG subclass concentrations in serum from 200 normal adults and IgG subclass determination of 106 myeloma proteins. An interlaboratory study. Scand J Clin Lab Invest. 1988; **48**:77.

59. Stein S F, Ware J H, Woods R: Serum immunoglobulins: Methods for the determination of normal values in international units. J Immunol Methods. 1977; **16**:371-384.

60. Yount W J, Dorner M M, Kunkel H, Kabat E: Studies on human antibodies. J Exp Med. 1968; **127**: 633-646.

61. Devey M E, Wilson D V, Wheeler A W: The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. Clin Allergy. 1976; **6**: 227-236.

62. Kraft D, Hedin H, Richter W, Scheiner O, Rumpold H, Devey M E: Immunoglobulin class and subclass distribution of dextran-reactive antibodies in human reactors and non reactors to clinical dextran. Allergy. 1982; **37**: 481-489.

63. Hammerstrom L, Granstrom M, Oxelius V, Person M A A, Smith C I E: IgG subclass distribution of antibodies against *Staphylococcus aureus* teichoic acid and alpha toxin in normal and immunodeficient donors. Clin Exp Immunol. 1984; **55**: 593-601.

64. Djurup R, Osterballe O: IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. Allergy. 1984; **39**: 433-441.

65. Aalberse R C, Gaag R, Leeuwen J: Serologic aspects of IgG4 antibodies. 1.Prolonged immunization results in an IgG4-restricted response. J Immunol.1983; **130**(2): 722-726.

66. van der Giessen M, Goeneboer-Kempers O: The subclasses of human IgG antibodies against tetanus toxoid. Clin Exp Immunol. 1976; **25**:117-121.

67. Sarnesto A, Ranta S, Vaananen P, Makela O: Proportions of immunoglobulin classes and subclasses in rubella. Clin Exp Immunol. 1984; **55**:671-676.

68. Linde G A, Hammarstrom L, Persson M A A, Smith C I E, Sundquist V A, Wahren B: Virus- specific antibody activity of different subclasses of immunoglobulins G and A and cytomegalovirus infections. Infect Immun. 1983; **42**:237-244.

69. Morell A, Roth-Wicky B, Skvaril F: Immunoglobulin G subclass restriction of antibodies against hepatitis-B surface antigen. Infect Immun. 1983; **39**:565-568.

70. Coleman R M, Nahmias A J, Williams S C, Phillips D J, Black C M, Reimer C B: IgG subclass antibodies to herpes simplex virus. J Infect Dis. 1985; **151**:929-936.

71. Vandvik B, Natvig J B, Norrby E: IgG1 subclass response of oligoclonal measles virus-specific IgG antibodies in patients with subacute sclerosing panencephalitis in a patient with multiple sclerosis. Scand J Immunol. 1977; **6**:651-657.

72. Kaschka W P, Klein G, Hilgers R, Skvaril F: Humoral immune response in Epstein-Barr virus infections. II.IgG subclass distribution in African patients with Burkitt's lymphoma and nasopharyngeal carcinoma. Clin Exp Immunol. 1984; **55**:14-22.

73. Klasse P J, Blomberg J: Distinct patterns of antibodies to HIV proteins in different IgG subclasses. J Inf Dis. 1987; **156** (6):1027-1030.

74. Khalife J, Guy B, Capron M, *et al*: Isotypic restriction of the antibody response to human immunodeficiency virus. AIDS Res Hum Retroviruses. 1988; **4**(1):3-9.

75. Bratt G, Waldenlind L, von Krogh G, Karlson A, Moberg L, Biberfeld G, Sandstrom E: A prospective study of immunoglobulin changes in relation to human immunodeficiency virus infection in a cohort of homosexual men. Scand J Immunol. 1989; **29**:589-595.

76. Muller F, Froland S S, Brandtzaeg P: Altered IgG subclass distribution in lymph node cells and in serum of adults infected with human immunodeficiency virus (HIV). Clin Exp Immunol. 1989; **78**:153-158.

77. Aucouturier P, Couderc L J, Gouet D *et al*: Serum immunoglobulin G subclass dysbalances in the lymphadenopathy syndrome and acquired immune deficiency syndrome. Clin. exp. Immunol. 1986;

63:234-240.

78. Broliden P A, Morfeldt-Mansson L, Rosen J, Jondal M, Wahren B: Fine specificity of IgG subclass response to group antigens in HIV-1 infected patients. Clin Exp Immunol. 1989; **76**:216-221.

79. Shackelford P G, Granoff D M, Nelson S J, Scott M G, Smith D S, Nahm M H: Subclass distribution of human antibodies to *Haemophilus influenzae*. J Immunol. 1987; **138**: 587-592.

80. Granoff D M, Shackeford P G, Suarez B K *et al*: *Haemophilus influenzae* type b disease in children vaccinated with type b polysacchride vaccine. N Eng J Med. 1986; **315**: 1584-1590.

81. Catty D, Jassin A, Hassan K, Raykundalia C: IgG subclasses in parasitic infestations. Monogr Allergy. 1986; **19**: 144-155.

82. Salimonu L S, Williams A I O, Osunkoya B O: IgG subclass levels in Malaria-infected Nigerians. Vox Sang. 1982; **42**:248-251.

83. Wahlgren W, Berzins K, Perlmann P, Persson M: Characterization of the humoral immune responses in *Plasmodium falciparum* malaria. Clin Exp Immunol. 1983; **54**: 135-142.

84. Martin A, Plebani A, Ravelli A *et al*: IgG subclass serum levels in juvenile chronic arthritis. Ann Rheum Dis. 1986; **45**: 400-404.

85. Rubin R L, Tang F L, Chan K L, Pollard K M, Tsay G, Tan E M: IgG subclasses of autoantibodies in systemic lupus erythematosus, Sjogren`s syndrome, and induced autoimmunity. Immunol. 1986; **137**: 2528-2534.

86. Rodgaard A, Nielsen F C, Djurup R, Somnier F, Gammeltoft S: Acetylcholine receptor antibody in myasthenia gravis: Predominance of IgG subclasses 1 and 3. Clin. exp. Immunol.1987; **67**: 82-88.

87. Gwynn,C.M; Morrison Smith,J., Leon,G.L., Stanworth,D.R: Role of IgG4 subclass in childhood allergy. Lancet 1978; **1**: 910-911.

88. Aalberse R C, van der Gaag R, van Leewen J: Serologic aspects of IgG4 antibodies.Prolonged immunization results in an IgG4-restricted response. J Immunol. 1983; **130**: 722-726.

89. Shakib F, Stanworth D R, Smalley C A, Brown G A: Elevated serum IgG4 levels in cystic fibrosis patients. Clin Allergy. 1976; **6**: 237-240.

90. Schur P H, Borel H, Gelfand E W, Alper C A, Rosen F S: Selective gamma-Globulin deficiencies in patients with recurrent pyogenic infections. N Eng J Med. 1970; 283: 631-634.

91. Stanley P J, Carbo G, Cole P J: Serum IgG subclasses in chronic

and recurrent respiratory infections. Clin Exp Immunol. 1984; **58:** 703-708.

92. Oxelius V A: Chronic infections in a family with hereditary deficiency of IgG2 and IgG4. Clin Exp Immunol. 1974; **17:** 19-27.

93. Soderstrom T, Soderstrom R, Avanzini A, Brandtzaeg P, Karlsson G, Hanson L A: Immunoglobulin G subclass deficiencies. Int Archs Allergy Appl Immunol. 1987; **82**:476-480.

94. Ouchterlony O: Diffusion in gel methods for immunological analysis. In Progress in Allergy, eds. Kallos P. and Walesman B.H., vol. VI,Basel-Kargen, 1958; 30-150.

95. Mancini G, Carbonara A O, Heremans J F: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry. 1965; **2**: 235-254.

96. Jefferies R, Reimer C, Skvaril F *et al*: Evaluation of monoclonal antibodies having specificity for human IgG subclasses:Results of an IUIS/WHO collaborative study. Immunol Letters. 1985; **10**:223-252.

97. Barre'-Sinoussi F, Cherman J C, Rey F *et al*: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunedeficiency syndrome (AIDS). Science. 1983; **220**:868-871.

98. Gallo R C, Salahuddin S Z, Popovic M *et al*: Frequent detection of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science. 1984; **224**:500-503.

99. Coffin J, Haase A, Levy J A *et al*: Human immunodeficiency viruses. Science. 1986; **232**:697.

100. Centres for Disease Control: Update on Acquired immune deficiency syndrome (AIDS)-United States. MMWR 1982; **31**:507-514.

101. Centres for Disease Control: Possible transfusion-associated acquired immune deficiency syndrome (AIDS)-California. MMWR 1982; **31**:652-654.

102. Centres for Disease control: *Pneumocystis carinii* pneumonia among persons with haemophilia A. MMWR 1982; **31**:365-367.

103. Friedman-Kien A, Lauberstein L, Marmor M *et al*: Kaposi's sarcoma and pneumocystis pneumonia among homosexual men-New York and California. MMWR 1981; **30**:305-308.

104. Quinn T C, Mann J M, Curran J W, Piot P: AIDS in Africa: An epidemiological paradigm. Science. 1986; **234**:955-963.

105. Piot P, Plummer F A, Rey M *et al*: Retrospective seroepidemiology of AIDS virus infection in Nairobi population. J Infect Dis.

1987; **155**(6): 1108-1112.

106. Curran J W, Jaffe H W, Hardy A M, Morgan W M, Selik R M, Dondero T J: Epidemiology of HIV infection and AIDS in the United States. Science. 1988; **239**:610-616.

107. Jawetz E, Melnick J L, Adelberg E A, Brooks G F, Butel J S, Ornston L N: Medical Microbiology. Eighteenth Ed. U.S.A. Appleton & Lange, Prentice-Hall International Inc. 1989; 512-539.

108. Gelderblom H R, Hausmann E H S, Ozel M, Pauli G, Koch M A: Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structural proteins. Virol. 1987; **156**:171-176.

109. Ratner L, Haseltine W, Patarca R *et al*: Complete nucleotide sequence of the AIDS virus,HTLV-III. Nature. 1985; **313**:277-283.

110. Lillehoj E P, Salazar F H R, Mervis R J, Raum M G, Chan H W, Ahmad N, Venkatesan S: Purification and structural characterization of the putative **gag-pol** protease of human immunodeficiency virus. J Virol. 1988; **62**(8):3053-3058.

111. Gaines H, von Sydow M, Sonnerborg A *et al*: Antibody response in primary human immunodeficiency virus infection. Lancet. 1987;

I:1249-1254.

112. Goudsmit J, DeWolf F, Paul D A *et al*: Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. Lancet. 1986; **II**:177-180.

113. Veronesse F M, Copeland T D, DeVico A L, Rahman R, Oroszlan S, Gallo R C, Sarngadharan M G: Characterization of highly immunogenic p66/p51 as the reverse transcriptase of HTLV-III/LAV. Science. 1986; **321**:1289-1291.

114. Gendelman H E, Theodore T S, Willey R, McCoy J, Adachi A, Mervis R J, Venkatesan S, Martin M A: Molecular characterization of a polymerase mutant immunodeficiency virus. Virol. 1987; **160**:323-329.

115. Weiss R, Teich N, Varmus H, Coffin J. RNA tumor viruses. Molecular biology of tumor viruses. Cold Spring Harbor Laboratory, 1984.

116. Steimer K S, Higgins K W, Powers M A *et al*: Recombinant polypeptide from the endonuclease region of the acquired immune deficiency syndrome retrovirus polymerase (pol) gene detects serum antibodies in most infected individuals. J Virol. 1986; 58(1):9-16.

117. Wong-Staal F, Shaw G M, Hahn B H *et al*: Genomic diversity of human T-Lymphotropic virus type III (HTLV-III). Science. 1985; **229**:759-762.

118. Dalgleish A G, Beverly P C L, Clapman P R, Crawford D H, Greaves M F, Weiss R A: The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature. 1984; **312**:763-767.

119. Palker T J, Clark M E, Langlois A J *et al*: Type specific neutralization of the human immunodeficiency virus with antibodies to **env**-encoded synthetic peptides. Proc Natl Acad Sci USA. 1988; **85**:1932-1936.

120. Kowalski M, Potz J, Basiripour L *et al*: Functional regions of the envelope glycoprotein of human immunodeficiency virus type-1. Science. 1987; **237**:1351-1355.

121. Bolognesi D P: Natural immunity to HIV and its possible relationship to vaccine strategies. Microbiological Sciences. 1988; **5**(8):236-341.

122. Gonzalez-Scarano F, Waxham M N, Ross A M, Hoxie J A: Sequence similarities between human immunodeficiency virus gp 41 and paramyxoviruses fusion proteins. AIDS Res Hum Retroviruses. 1987; **3**(3):245-250.

123. Norrby E, Biberfeld G, Chiodi F, von Gegerfeldt A, Naucle'r A, Parks E, Lerner R: Discrimination between antibodies to HIV and related retroviruses using site-directed serology. Nature. 1987; **239**:248-250.

124. Haseltine W A. Replication and pathogenesis of the AIDS virus: J AIDS 1988; **1**:217-240.

125. Klatzman D, Champagne E, Chamaret S *et al*: T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature. 1984; **312**:767-768.

126. Ho D D, Kaplan J C, Rackauskas I E, Gurney M E: Second conserved domain of gp120 is important for HIV infectivity and antibody neutralization. Science. 1988; **239**:1021-1023.

127. Weber J N, Weiss R A: HIV infection: the cellular picture. Sci Amer. 1988; October, 101.

128. Fauci A S: The human immunodeficiency virus: Infectivity and Mechanisms of pathogenesis. Science. 1988; **239**:617-622.

129. McKeating J A, Griffiths P D, Weiss R A: Susceptibility conferred to human fibroblasts by cytomegalovirus-induced Fc receptor. Nature. 1990; **343**:659-661.

130. Harouse J M, Kunsch C, Hartle H T, Laughlin M A, Howie J A, Wigdahl B, Gonzales-Scarmo F: CD4-independent infection of human neural cells by human immunodeficiency virus type I. J Virol. 1989; **63**:2527-2533.

131. Bowen D L, Lane H C, Fauci A S: Immunopathogenesis of the acquired immunodeficiency syndrome. Ann Int Med. 1985; **103**:704-709.

132. Fauci A S: Immunologic abnormalities in AIDS. Clin Res. 1985; **32**:491-499.

133. Fauci A S: AIDS: Immunopathogenic mechanisms and research strategies. Clin Res. 1987; 35:503-510.

134. Melcher G P: AIDS in Arkansas. Update: The immunopathogenesis of human immunodeficiency virus. J Arkansas Med Soc. 1989; 85(11):479-483.

135. Sodroski J, Goh W C, Rosen C, Campbell K, Haseltine W A: Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature. 1986; 322:470-474.

136. Lifson J D, Reyes G R, McGrath M S, Stein B S, Engleman E G: AIDS retrovirus induced cytopathology: Giant cell formation and involvement of CD4 antigen. Science. 1986; **232**:1123-1126.

137. Ho D D, Pomerantz R J, Kaplan J C: Pathogenesis of infection with human immunodeficiency virus. N Engl J Med. 1987; **317:**(5) 278-286.

138. Lane H C, Deeper J M, Greene W C, Whalen G, Waldmann T A, Fauci A S: Quantitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. N Engl J Med. 1985; **313**(2):79-84.

139. Bernidge M J, Irvine R F: Inositol triphosphate, a second messenger in cellular signal transduction, Nature. 1984; **312**:315-321.

140. Quinnan G V, Siegel J P, Epstein J S, Manischewitz J F, Barnes S, Wellis M A: Mechanisms of T-cell functional deficiency in the acquired immunodeficiency syndrome. Ann Int Med. 1985; **103**:710-714.

141. Poli G, Bottazzi B, Acero R *et al*: Monocyte function in intravenous drug abusers with lymphadenopathy syndrome and in patients with acquired immunodeficiency syndrome: selective impairment of chemotaxis. Clin Exp Immunol. 1985; **62**:136-142.

142. Lane H C, Masur H, Edgar L C, Whalen G, Rook A H, Fauci A S: Abnormalities of B-cell activation and immunoregulation in

patients with the acquired immunodeficiency syndrome. N Engl J Med. 1983; **309**(8): 453-458.

143. Schnittman S M, Lane H C, Higgins S E, Folks T, Fauci A S: Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. Science. 1986; **233**:1084-1086.

144. Pahwa S G, Quilop M-T J, Lange M, Pahwa R N, Grieco M H: Defective B-lymphocyte function in homosexual men in relation to the acquired immunodeficiency syndrome. Ann Int Med. 1984; **101**:757-763.

145. Casareale D, Sinangil F, Hedeskog M, Ward W, Volsky D J, Sonnabend J: Establishment of retrovirus-,Epstein-Barr virus-positive B-lymphoblastoid cell lines derived from individuals at risk for acquired immune deficiency syndrome (AIDS). AIDS Res. 1984; **1**:253.

146. Montagnier L, Gruest J, Chamaret S, *et al*: Adaptation of lymphadenopathy Associated Virus (LAV) to replication in EBV-transformed B lymphoblastoid cell lines. Science. 1984; **225**:63-66.

147. Yarchoan R, Redfield R R, Broder S: Mechanisms of B cell activation in patients with acquired immunodeficiency syndrome

and related disorders. Contribution of antibody-producing B cells, of Epstein-Barr virus-infected cells, and of immunoglobulin production induced by human T cell lymphotropic virus type III/Lymphadenopathy-associated virus. J Clin Invest. 1986; **78**:439-447.

148. Chirmule N, Kalyanaraman V S, Saxinger C, Wong-Staal F, Ghrayeb J, Pahwa S: Localization of B-cell stimulatory activity of HIV-1 to the carboxyl terminus of gp41. AIDS Res and Hum Retroviruses. 1990; **6**(3):299-305.

149. Mizuma H, Zolla-Pazner S, Litwin *et al*: Serum IgD elevation is an early marker of B cell activation during infection with the human immunodeficiency viruses. Clin Exp Immunol. 1987; **68**:5-14.

150. Mathiesen T, Persson M A A, Sundqvist V.-A, Wahren B: Neutralization capacity and antibody dependent cell-mediated cytotoxicity of separated IgG subclasses 1, 2, 3 and 4 against herpes simplex virus. Clin Exp. Immunol. 1988; **72**:211-215.

151. Sundqvist V.-A, Linde A, Kurth R, Werner A, Helm E B, Popovic M, Gallo R C, Wahren B: Restricted IgG subclass responses to HTLV-III/LAV and to cytomegalovirus in patients with AIDS and lymphadenopathy syndrome. J Infect Dis. 1986; **153**(5):970-973.

152. Chiodi F, Mathiesen T, Albert J, Parks E, Norrby E, Wahren B:

IgG subclass responses to a transmembrane protein (gp41) peptide in HIV infection. J Immunol. 1989; **142**(11):3809-3814.

153. Stanworth D R, Solder B, Lewn I V, Nayyar S, Marschang P, Wachter H, Dierich M P: Related epitopes in HIV viral coat protein and human IgG. Lancet. 1989; 1258-1459.

154. Robinson W E Jr, Montefiori D C, Mitchell W M: Antibody-dependent enhancement of human immunodeficiency virus type-1 infection. Lancet. 1988; **i**:790-794.

155. Weinhold K J, Lyerly H K, Mathews T J *et al*: Cellular anti-gp120 cytolytic reactivities in HIV-1 seropositive individuals. Lancet. 1988; **i**:902-903.

156. Shepp D H, Chakrabarti S, Moss B, Quinnan G V Jr: Antibody-dependent cellular cytotoxicity specific for the envelope antigens of human immunodeficiency virus. J Infect Dis. 1988; **157**(6):1260-1264.

157. Tyler D S, Lyerly H K, Weinhold K J: Minireview anti-HIV-1 ADCC. AIDS Res Hum Retroviruses. 1989; 5(6):557-563.

158. Walker C M, Moody D J, Stites D P, Levy J A: CD8+ Lymphocytes can control HIV infection *in vitro* by suppressing virus replication. Science. 1986; **234**:1563-1567.

159. Walker B D, Flexner C, Paradis T J, Fuller T C, Hirsch M S, Schooley R T, Moss B: HIV-1 Reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals. Science. 1988; **240**:64-66.

160. Garrett A J, Seagroatt V, Supran E M, Heberehl K O, Hampl H, Schild G C: Measurement of antibodies to human immunodeficiency virus: An international collaborative study to evaluate WHO reference sera. PAHO Bull. 1989; **3**(1-2):207-215.

161. Harry D J, Jennings M B, Yee J, Carlson J R: Antigen detection for human immunodeficiency virus. Clin Microbiol Rev. 1989; **2**(3):241-249.

162. World Health Organisation: Acquired immunodefiency syndrome (AIDS). Wkly Epidem Rev. 1986; **61** :69-73.

163. WHO Wkly Epidem Rev. 1990, **65**(37):281-283.

164. Rowe D S, Grab B, Anderson S G: An international reference preparation for human serum immunoglobulins G, A, and M:Content of immunoglobulin by weight. Bull WHO. 1972, **46**:67.

165. Hamilton R G, Wilson R W, Spillman T, Roebber M: Monoclonal antibody-based immunoenzymetric assays for quantification of human IgG and its four subclasses. Journal of Immunoassay. 1988;

9(3&4):275-296.

166. Litwin S D, Tse-Eng D, Butler V P, Perrais B: Studies on human low serum IgD phenotype and Gm markers. Clin Genetics. 1985; **27**:134.

167. Lucey D R, Zajac R A, Melcher G P, Butzin C A, Boswell R N: Serum IgE levels in 622 persons with human immunodeficiency virus infection:IgE elevation with marked depletion of CD4+ T-cells. AIDS Res and Hum Retroviruses. 1990; 6(4):427-429.

168. Dunnete L S, Glick G J, Miller R D, Kyle R A: Measurement of IgD by a double antibody radioimmunoassay: demonstrations of an apparent trimodal distribution of IgD levels in normal human sera. J Immunol. 1977; **119**:1427.

169. Black C M, Plikaytis B D, Wells T W, Ramirez R M, Carlone G M, Chilmonczyk B A, Reimer C B: Two-site immunoenzymometric assays for serum IgG subclass infant/maternal ratios at full term. J Immunol Methods. 1988; **106**:71-81.

170. Bahma S L, Heiner D C, Horwitz C A: Sequential changes of the five immunoglobulin class and other responses in infectious mononucleosis. Int Archs Allergy Appl Immunol. 1984; **74**:1.

171. Buckley C E, Trayer H R: Serum IgD concentrations in sarcoidosis

and tuberculosis. Clin Exp Immunol. 1972; **10**:257.

172. Nardbring F, Hagman C, Johanson S G O: Serum immunoglobulin levels in the course of acute pneumonia. Scand J infect Dis. 1969; **1**:99.

173. Romagnani S, Del Prete G F, Maggi E, Bellesi G, Biti G: Abnormalities of *in vitro* immunoglobulin synthesis by peripheral blood lymphocytes from patients with Hodgkin's Disease. J Clin Inv. 1983; **71**:1375.

174. Zolla-Pazner S. B cell in the pathogenesis of AIDS: Immunol Today. 1984; **5**:289.

175. Procaccia S, Lazzarin A, Colucci A *et al*: IgM, IgG and IgA rheumatoid factors and circulating immune complexes in patients with AIDS and AIDS-related complex with serological abnormalities. Clin Exp Immunol. 1987; **76**(2):236-244.

176. Martinez-Maza O, Crabb E, Mitsuyasu R T, Fahey J L, Gorgi J V: Infection with the human immunodeficiency virus (HIV) is associated with an *in vivo* increase in B lymphocyte activation and immaturity. J Immunol. 1987; **138**:3720-3724.

177. Mizuma H, Litwin S, Zolla-Pazner S: B-cell activation in HIV infection: relationship of spontaneous immunoglobulin secretion

to various immunological parameters. Clin Exp Immunol. 1988; **71**:410-416.

178. Forsgren A, Grubb A O: Many bacterial species bind human IgD. J Immunol. 1979; **122**:1468.

179. Carini C, Margolick J, Yodoi J, Ishizaka K: Formation of IgE-binding factors by T-cells of patients infected with human immunodeficiency virus type 1. Proc Natl Acad Sci USA. 1988; **85**:9214.

180. Eisenberg R A, Dyer K, Craven S Y, Randall Fuller C, Yount W J: Subclass restriction and polyclonality of the systemic lupus erythematosus marker antibody anti-Sm. J Clin Invest. 1985; **75**:1270.

181. Taele J M, Abraham K M: The regulation of antibody class expression. Immunol Today. 1987; 8:122-126.

MED. THS
RC 607
.A26
L92
1991
Copy 1