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INTRAVENOUS IMMUNOGLOBULIN PROPHYLAXIS OF LATE-ONSET SEPSIS IN PRETERM NEONATES

By

MUFIDA MOHAMMED EL-BELAZI

Thesis submitted for the degree of M.Sc. (Med. Sci.)

Department of Child Health, Faculty of Medicine,

University of Glasgow

1995

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ABSTRACT

Advances in neonatal care during the past 15 years have resulted in an increased survival rate for preterm newborn infants with birth weight of less than 1500 grams. Despite therapeutic improvements, infection remains a major problem in this group of babies due to their increased susceptibility to infection.

In a double blind, placebo controlled trial, we investigated the effect of intravenous immunoglobulin (IgG) in the prevention of nosocomial infection in very low birth weight infants (with birth weight $\leq$ 1500 grams and gestational age $\leq$ 32 weeks).

Patients were recruited from the Neonatal Intensive Care Unit at the Glasgow Royal Maternity Hospital. Thirty eight infants were enrolled into the trial and all needed intensive life support and/or parenteral nutrition through a long line.

After randomisation 19 were given IVIG and 19 were used as controls. Four babies died early in the study and were excluded.

The babies were intensively monitored during the study period for the number of proven or suspected episodes of infection and any complication of therapy. Additional information about their ability to resist infection was obtained by checking their opsonic activity.

Babies $\leq$ 1000 grams birth weight were given 700 mg/kg of IVIG. Babies with 1001 - 1500 grams were given 500 mg/kg of IVIG. The placebo given to control group was 5 % dextrose. These doses were repeated at two weekly intervals so long as the babies needed intensive care.

We conclude that the administration of IVIG did not provide effective prophylaxis for late-onset sepsis. IVIG appears to be well tolerated by very low birth weight infants. No significant side effects were observed.

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ABBREVIATIONS

F.F.P.	Fresh frozen plasma
IVIG	Intravenous immunoglobulin G
PVH/IVH	Periventricular intraventricular haemorrhage
SVD	Spontaneous vertex delivery
GBS	Group B β- haemolytic streptococci
PMN	Polymorphonuclear neutrophil
LBW	Low birth weight
VLBW	Very low birth weight
IVIG	Intravenous immunoglobulin
CFU-GM	Colony forming unit- granulocyte macrophage
NEC	Necrotizing enterocolitis
LUSCS	Lower uterine segment caeserian section
PROM	Premature rupture of membrane
CFU	Colony forming unit
CRP	C- reactive protein
UAC	Umblical arterial catheterisation
UVC	Umblical venous catheterisation

Chapter 1

1.1 Introduction and Aim of the study

Advances in neonatal care during the past 15 years resulted in an increased survival rate for preterm new-born infants, particularly those weighing less than 1500 grams at birth[1,2]. As a result, a considerable number of very low birth weight infants stay for up to several months in neonatal intensive care units, exposed to various invasive procedures and they are susceptible to serious nosocomial infections. The majority of nosocomial infections in these neonatal units occur in preterm infants with an attack rate of up to 20%[3]. During the hospital stay which may range from a few weeks to several months, many preterm infants will experience more than one episode of nosocomial infection. Increased susceptibility to nosocomial infections in preterm infants is multifactorial. Conditions associated with risk include disruption of mucosal and skin barriers during invasive procedures such as intubation, umbilical arterial and venous catherisation, exposure to nosocomial pathogens, multiple courses of broad-spectrum antibiotics, and immaturity of host defence mechanisms.

Immaturity of host defence mechanisms result in a reduced humoral immunity. B lymphocytes do not mature to become plasma cells and produce IgG. This together with the fact that preterm infants are born with low serum IgG concentrations contributes to very low levels of circulating IgG [4]. During the first weeks of life the deficiency of transplacentally acquired specific antibody causes an increased risk to the infants of certain neonatal infections, such as those caused by group B β-haemolytic streptococci and Escherichia coli capsular serotype K1[5].

Other factors affecting humoral immunity include complement and fibronectin. These are necessary for opsonization of bacteria and are present in considerably reduced concentrations in infants born prematurely. Cellular immunity is also defective in preterm infants. Quantitative and qualitative deficiency in the myeloid and phagocyte cells lead to inability of these cells to respond to specific bacterial antigens so that the infants ability to respond to bacterial infections is further compromised.

During the last decade there have been a number of attempts to enhance the neonatal host defence mechanisms by using granulocyte transfusions to provide improved cellular immunity and opsonization. Exchange transfusion with fresh donor blood, fibronectin transfusion to foster neutrophil adhesion and opsonization, and the use of intravenous immunoglobulin are therapeutic strategies which have been used in an attempt to improve opsonization and bacterial killing.

Most neonatal infections are bacterial in origin and most reported studies have focused on the use of intravenous immunoglobulin (IVIG) in an attempt to prevent or to reduce the severity of neonatal infections.

Some studies have shown a beneficial effect of IVIG in prophylaxis and in therapy whilst others have failed to demonstrate any benefit. A variety of outcome measures such as the incidence of culture proven sepsis, a reduced mortality and a reduction in the duration of stay in hospital were used in these intervention studies but there remains considerable uncertainly about the effectiveness of IVIG.

In developing countries neonatal bacterial infections are a major cause of death and severe handicap in those who survive. If IVIG were to be shown to prevent or significantly reduce neonatal sepsis there would be a major health benefit to be gained.

The present study has been set up in order to explore further the value of IVIG in the prevention of bacterial infections in very low birth weight infants.

1.2 Neonatal Infection

Neonatal infection is an important cause of morbidity and mortality in new-born infants especially those born prematurely. Neonatal bacterial sepsis occurs in approximately 1 to 10 per 1000 live births and is increased almost fourfold in preterm babies[6,7]. Advances in intensive care over the last two decades have resulted in improved survival of babies born before 32 weeks gestation and/or weighing under 1500 grams. Because these babies stay in a hospital for several weeks prior to dismissal there is a 15-30%[8] risk of late onset nosocomial infection.

This is the result of multiple risk factors which including immaturity of host defence mechanisms, endotracheal intubation, arterial or venous umbilical catheterization, the insertion of long lines which result in disruption of mucosal and skin barriers and multiple courses of antibiotic. The incidence of infection in these infants is about 24-32%[9,10]. Despite the development of new broad spectrum antibiotics, the mortality rate has remained unacceptably high and ranges from 11 to 33% and as high as 20% to 75%[6,11] in these babies who are neutropenic.

The incidence of neonatal sepsis varies among hospitals due to the differences in patient population with respect to risk factors. Two studies[8,12] reported that the incidence of neonatal sepsis were under estimated. They reported that bacteria were isolated from the blood of more than 40 % of babies who died during the first twenty eight days of life and that in two thirds of the 40%, post-mortem cultures provide the only evidence of infection.

The morbidity and mortality associated with neonatal sepsis is inversely related to gestational age, VLBW infants exhibiting a higher mortality than full term infants. They are also pathogen specific, with sepsis due to group B streptococci resulting in a higher mortality than sepsis due to staphylococci. The outcome of neonatal sepsis is also dependent on the immunological and physiological status of the baby at the time of presentation, the presence of perinatal risk factors, the use of invasive supportive life measures, early diagnosis and treatment with appropriate antibiotic therapy and the management of metabolic and respiratory acidosis[6,11]. Further, sepsis associated with hypotension, disseminated intravascular coagulation and other organ dysfunction is associated with a mortality that approaches 90% in the VLBW infants[11].

1.3 Predisposing Factors of neonatal Infection

There are multiple risk factors which predispose premature infants to neonatal infection. These can be grouped into two groups: maternal and neonatal.

A. Maternal risk factors

1. Prolonged rupture of membranes and chorioamnionitis

Many studies[13,14] have shown that the incidence of documented sepsis in the neonate born to mothers with rupture of membranes for more than 24 hours is approximately 1%. With signs and symptoms of chorioamnionitis the risk of sepsis increases to 3-5%.

Boyer et al.[15] studied the correlation between the duration of rupture of the membranes and clinical neonatal infection with GBS and noted the attack rate ranged from 0.8 per 1000 live births when the membrane has ruptured for less than 6 hours, up to 10.8 per 1000 live births if the membranes were ruptured for more than 24

hours. Over all, the relative risk of developing early onset disease was 7.2 times higher when the membranes were ruptured for more than 18 hours before birth. Intrapartum fever >37.5 °C increases the sepsis rate to 6.5 per 1000 live births compared with 1.5 per 1000 live births in women who were afebrile. The placental pathogenic diagnosis is easy to make, but that result is rarely known in time to influence clinical management.

2. Maternal colonisation

Maternal colonisation with GBS without other clinical complications carries a risk of neonatal sepsis of 0.5% to 1%[16]. Materno-fetal transmission can occur from an ascending route in utero or during birth through a colonised birth canal. The vertical transmission rate ranged from 42% to 72% among infants delivered by colonised women[17]. Sepsis rates for clinical disease are low in colonised neonates and range from 0.7 to 3.7 per 1000 live births during the first week of life, and 0.5 to 1.7 per 1000 live births after 7 days of life.

Another study[18] found the sepsis rate during the perinatal period in infants born to GBS colonised mothers was 1% to 2% but increased to 15.2% with premature onset of labour of <37 weeks, to 10.7% with chorioamnionitis or PROM of >24 hours and to 9.7% with maternal post partum bacteraemia[19].

Twin gestation may be at increased risk for GBS sepsis when associated with prematurity[18,20]. One explanation may be that GBS infection of a previous sibling may be a risk factor[21]. Presumably the mothers of these babies do not have sufficient GBS antibody to pass to their fetus or deliver a preterm baby that has not been in utero long enough to receive protective antibody. A major limitation to the ability to manage GBS infection postnatally is that the majority of early onset disease is acquired in utero.

3. Maternal urinary tract infection

Unless treated and resolved before labour, urinary tract infection is associated with an increased risk of infection in the neonate because of an increased risk of preterm delivery and chorioamnionitis[21].

B. Neonatal risk factors

1. Prematurity and low birth weight

The preterm baby is deficient in most arms of the immune system including immunoglobulin, complement, fibronectin, opsonic function and phagocytic capacity of WBC[22]. Colonisation rates with GBS are similar between preterm and term infants but the rate of GBS infection is considerably higher in preterm infants. Following PROM the rate of infection in the preterm infant is 8 to 10 times higher than in the term infant, these infants exhibiting a sepsis rate ranging from 4% to 11%[14,23]. Boyer et al.[15] prospectively studied 61 infants with GBS infection. They noted a high sepsis rate among infants weighing less than 2500 gram, 7.6 per 1000 live births in comparison to 1.1 per 1000 live births in term infants. In another study[24] the rate of sepsis was 8 times greater in infants weighing 1000 - 1500 gram compared with those weight 2000 - 2500 gram. Meningitis occurred 3 - 17 times more often in those weighing less than 2500 gram compared with those weighing more than 2500 gram.

2. Perinatal asphyxia

In a study by Geme et al.[14] a 5 minute Apgar score of <6 in the presence of PROM was a useful predictor of neonatal sepsis as choriamnionitis. A Danish study[25] found that 27% of preterm infants with PROM and perinatal asphyxia had proven sepsis.

3. Male gender

Observation for many years confirms that male infants are 2-6 times more likely to develop perinatal sepsis than female infants[14,26]. The reasons for this finding have not been elucidated.

4. Traumatic delivery

Forceps and vacuum delivery carry a great risk of trauma, skin aberration and birth asphyxia.

1.4 Microbiology

1. Group B beta haemolytic streptococci [GBS]

This is the most common cause of neonatal sepsis and meningitis in the United States with an incidence of 2-4 per 1000 live births. It most commonly occurs during the first few days of life (early onset disease) with a mean onset of 20 hours[27]. It is acquired from infected amniotic fluid or swallowed inoculum during transit through the vaginal canal. The carriage rate at delivery for mothers was 20% and for new-born 12%. The rate of early onset disease is higher in infants found to be heavily colonised at birth. Preterm delivery, prolonged labour, premature rupture of membranes and maternal infection enhance the risk of early disease. Late onset disease occurs after the first week of life with a mean onset of 24 days. The organisms may be acquired from the mother or nosocomially. It has an incidence of 1-2 per 1000 live births. Mortality rate from GBS infection varies from 20% to 50% and is more common in preterm infants.

The type III strain of capsular serotype of GBS is the main organism causing disease in the neonate. GBS has a type specific polysaccharide which protects it from phagocytosis, unless the organism is opsonised (which involves the deposition of

specific antibody and/or complement on the bacterial surface). Neutropoenia is common in GBS disease occurring in up to 30% of affected neonates. It is a poor prognostic sign with a mortality rate of 50% - 75%.

2. Escherichia coli K1

The overall incidence of E-coli infection is estimated to be 1-2 per 1000 live births. The majority of these infections are caused by strains possessing the K1 polysaccharide capsule[28]. The intestine of new-born infants, which is sterile in utero, becomes colonised by E-coli shortly after birth. The colonising strains originate from the maternal faecal flora or are acquired from the environment.

3. Listeria monocytogenes

It is difficult to know the true incidence of Listeria infection. One study in the United States between 1980-1983 suggests that the incidence of neonatal infection may be as high as 0.6 per 1000 live births[29] with a mortality rate of 19.1%.

4. Haemophilus influenzae [HI]

Infection with HI occurs usually in utero or in the immediate post-partum period. It is associated with prematurity in 83%. The overall mortality rate is 55% and may be as high as 90% in preterm neonates of less than 30 weeks gestation[30].

5. Nosocomial infection

Coagulase negative staphylococci have become an important cause of nosocomial bacteraemia in recent years. This may be due to a change in the nursery population with an increased proportion of very low birth infants. It accounts for more than 50% of bacteraemia in neonatal intensive care units. Neonatal sepsis and meningitis with coagulase negative septicaemia are associated with significant morbidity and a mortality rate as high as 10%[31].

Coagulase negative staphylococci are the most common infective agent causing septicaemia after 48 hours of age. Nosocomial infection is a significant problem for neonates requiring prolonged care in the nursery especially in the case of very low birth infants. The overall incidence has been reported to be as high as 15% to 30% in babies whose birth weight was less than 1500 grams[31]. Beyond the first week of life, the neonate who remains in special care is likely to be colonised with endogenous and nosocomially acquired flora especially coagulase negative staphylococci, Staphylococcus aureus and other organisms causing late onset disease such as GBS and Listeria. The strains of coagulase negative staphylococci most frequently associated with neonatal infection are often resistant to a wide range of antibiotics and are usually spread from patient to patient on the hands of nursing and medical staff.

The most frequently identified factors contributing to nosocomial infection are the length of stay in nursery, low birth weight, foreign bodies (intravascular catheter, chest drain and endotracheal tube), nursery crowding, surgery and prolonged treatment with broad spectrum antibiotics. The number of bacteria on the skin surface will determine the probability and degree of contamination of the catheter at the time of insertion. Local immune defences are more likely to be overcome when large numbers of bacteria are present on the skin at the catheter insertion site.

Many strains of coagulase negative staphylococci produce a complex mucopolsaccharide which has been termed extracellular slime substance. Electron microscopy studies suggest that it stabilises the attachment of coagulase negative staphylococci to the surface of foreign bodies such as intravascular catheters. Extracellular slime substance has been reported to have a number of immunomodulating effects such as inhibiting antibody binding the staphylococcal cell wall, reducing the chemotactic response of neutrophils and interfering with T and B cell function. Although extracellular slime substance is probably important in

stabilising the attachment of bacteria on surfaces, its importance in determining pathogenicity is controversial.

Gram positive organisms (S. aureus, S. epidermidis, group D streptococci) account for 56% of nosocomial sepsis whereas gram negative bacteria represent 25% and candida species another 13%.

1.5 Pathogenesis of bacterial infection

In utero the fetus lives in a sterile environment and becomes colonised and occasionally infected when he/she comes into contact with vaginal flora. During the postnatal period the neonate may acquire infection from various sources including hospital staff, mother and other infants, and exposure to equipment heavily contaminated with bacteria. Equipment requiring humidification such as incubators or respiratory equipment is commonly contaminated with water loving organisms. Techniques that have proved life saving for sick or small neonates may also increase the risk of infection by breaching the mucocutaneous barrier to infection.

There are three lines of defence mechanisms that protect the infants from infection.

1.5.1 Physical defence systems

At the surface of the body and within body cavities that communicate with the surface (respiratory tract, genitourinary tract and gastrointestinal tract) there are anatomic barriers and fluids that inhibit attachment and invasion of organisms. Among these the skin is the greatest natural barrier to infection. Exclusive of the resident, commensal bacteria, most micro-organisms are inactivated by material on the skin

surface such as fatty acids. During the neonatal period, the skin is functionally and anatomically immature, permeability is increased and there is increased susceptibility to blister formation in the neonate[32].

The pH of the skin is higher during the first few days of life than in the older child or adult[33]. Quantitatively the skin surface lipids are similar to those in the older children. However during the first two weeks of life cholesterol is decreased and wax esters increase. The skin's free fatty acids and triglyceride slowly decrease during the post natal period[34]. Postnatally, the skin of the new-born infant becomes extensively colonised, and breaks in the skin integrity (scalp sampling, umbilical catheterisation and forceps trauma) may allow the micro-organisms to enter the body, thus increasing susceptibility to infection. The umbilical cord, provides an excellent medium for bacterial growth and acts as a potential source of sepsis due to its proximity to the blood stream.

Immature gastrointestinal host defences may permit aberrant bacterial colonisation resulting in inadequate neutralisation of bacterial toxins and allows bacteria and toxins to gain access to intestinal tissue. New-born infants and especially preterm infants have lower rates of gastric acid secretion than older infants. Such hypochlorhydria is associated with bacterial colonisation of the stomach in the majority of enterally fed preterm infants[35,36]. Gastric acidity provides the first line of defence against bacterial colonisation of the upper gastrointestinal tract. A relatively high gastric pH may also inhibit protein digestion and permit the passage of intact molecules into the small intestine[37]. Proteolytic enzymes produced by the pancreas probably play an important role in limiting the exposure of the small intestine to toxins and other intact protein molecules. New-borns have a much lower level of tryptic activity in their intestinal fluid than older infants[38], resulting in impaired protein

digestion and bacterial toxin destruction. There is also reduced biosynthesis of bile acids in the new-born which has an important antibacterial implication.

Poor intestinal motility may also lead to stasis and bacterial over-growth. Duodenal peristaltic patterns are markedly immature until approximately 29 weeks gestation after which they begin to assume more mature patterns[39]. New-born infants also have specific gastrointestinal immunological deficiencies including a reduced number of T cells in their intestinal mucosa. This results in an inability to destroy infected intestinal epithelium cells. Stool levels of IgA are quite low in new-borns compared with older infants and children[40]. Secretory IgA appears to play a role in host defence mechanisms by complexing with antigen in the intestinal lumen, thus preventing its access to intestinal tissue. There is evidence that the preterm intestine is more permeable to carbohydrate, protein and bacteria than in older infants.

In preterm infants the development of respiratory bronchioles, alveolar ducts and sacs is incomplete. An immature ciliary apparatus leads to sub-optimal removal of inflammatory debris, mucus and bacteria. The neonatal lungs also have an insufficient number of pulmonary macrophages for intrapulmonary bacterial clearance. Other natural barriers to infection, including the mucociliary blanket and filtering mechanism of the nose are immature in preterm infants. Many premature neonates require respiratory support and during this time the respiratory tract rapidly becomes colonised with bacteria and fungi. This results in an increased incidence of systemic infection. Antibodies in the secretions play an important part in protecting the infants from infection by preventing adherence of antigen and by neutralising the organisms and their products. Forysth et al.[41] have shown that the concentration of IgA and IgM in preterm infants tracheo-bronchial secretions increases with increasing post-natal age. Intubated preterm neonates have been shown to produce IgM antibody to bacteria present in the lower respiratory tract. Robertson et al.[42] suggested that very

preterm infants are apparently able to acquire mucosal IgA and specific antibody but at a lower rate than gestationally older infants. This suggests slower postnatal maturation of mucosal antibody transport and production than in those born at full term.

1.5.2 Cellular immunity

A normal adult produces about 130 billion neutrophils per day from the bone marrow. Polymorphonuclear (PMN) leukocytes originate from pleuripotent stem cells in the bone marrow. They begin as myeloblasts which differentiate by division into myelocytes in about one week. During a 7 days post-mitotic period cytoplasmic and nuclear changes occur that result in a fully mature and functional PMN leukocyte. The bone marrow stores these mature neutrophils, so that they can be called upon under conditions of stress or infection. This reserve is about 13 times greater than the numbers of these cells in the circulation. The PMN leukocytes acquire two major populations of granules during development. The primary azurophilic granules appear during the mitotic period and contain myeloperoxidase, acid hydrolases, lysozyme cationic protein and neutral proteases. Primary granules are released into the phagosome following phagocytosis and provide the PMN with localised microbicidal activity.

The secondary (specific) granules are formed during the 7 days maturation period and contain lactoferrin, lysozyme, vitamin B12 binding protein, cytochome b, collagen, and certain receptor molecules. The specific granules which discharge much of their contents out of cells are thought to provide a regulatory function in inflammatory response. A third type of granule has been identified. It contains gelatinase, cytochrome b, and MAC-1 glycoprotein receptors which have been identified as an important mediator of adherence. Circulating PMN leukocyte

represent only 5% of the body pool. Neutrophils appear to have only a very short half life of about six to eight hours in the vascular pool. They are equally and randomly distributed between a population of marginal cells which are adherent to endothelial layer of blood vessels and a population of circulating cells. When PMN leukocyte enter the tissue they remain functional for 1 to 2 days. Senescent PMN leukocyte are removed by splenic macrophages or discharged from mucosal surface. An increase in both total numbers and percentage of circulating PMN leukocyte can be induced by acute infection, endotoxins and steroid. This increase occurs via increased bone marrow production, accelerated bone marrow release and demargination. In case of an acute inflammation the blood flow through an area of acute inflammation is markedly increased, the vascular endothelium becomes sticky and the contained neutrophils attack to it and then push their way between the endothelial cells. Once outside the capillary, the accumulation of these cells results from their positive migration toward the chemoattractants like bacterial products, autologus debris, the cleavage products of the third component of complement (C3a) and the granule contents from mast cells.

i. Normal blood leukocytes in the fetus and new-born infant

Many studies have been done to establish comprehensive reference ranges of total and differential leukocyte counts in the human fetus. Davies et al.[43] studied the total and differential leukocyte counts from 18 weeks of gestation to term (obtained from cordocentesis and elective Caesarian section). They found that the total leukocyte count in the fetus increased with progress of gestation, from 2.27 x 10^9/l at 18 weeks to 9.41 x 10^9/l at 40 weeks. This increase reflects two components: a linear increase in the lymphocyte count from a mean of 1.17 x 10^9/l at 18 weeks of gestation to 3.67 x 10^9/l at 40 weeks, together with an exceptional rise in neutrophil count from a mean value of 0.77 x 10^9/l at 31 weeks to 6.53 x 10^9/l at 40 weeks. Monocyte number increased linearly with gestation from a mean of 0.07 x 10^9/l at 18 weeks to

$0.44 \times 10^9/l$ at 40 weeks. These changes were paralleled by a reciprocal decrease in the erythroblast count.

No significant increase in eosinophil count was noted with progress of gestation. Early myeloid cells (metamyelocyte, promyelocyte, myelocyte, blast cells) were observed in 24% of total films and basophils in 15%. They contributed less than 2% of the total white cell count and there was no significant change with gestational age.

In intrauterine life the total leukocyte count increases with gestational age until 37-38 weeks gestation when lymphocytes predominate. However from 32 weeks onwards the proportion of neutrophils increases to become the commonest leukocyte at term. The changes in the number of circulating leukocytes as well as changes in other fetal haematological values with gestation were also noted in other studies[44,45]. This reflects an alteration in the pattern of pleuripotent haemopoietic stem cells to meet changing fetal physiological priorities during gestation.

The placenta acts as an effective barrier to most bacteria. Therefore the acquisition of a host defence mechanism directed against bacterial infection is only necessary in preparation for extrauterine life. This may explain the dramatic rise in the number of circulating neutrophils during the late third trimester. After birth there are also some changes. Neutophil concentration is quite high at birth and may rise even higher during the first days of life. By two weeks the neutrophil count declines to within and occasionally below normal levels.

ii. Function of PMN Leukocyte

When bacteria invade the tissue, an acute inflammatory response occurs, PMNs become activated and large numbers are brought to the site of inflammation. PMNs

adhere to the vessel wall. This is enhanced by cell surface glycoprotein then they pass through the capillary wall to interstitial tissue. After migration PMNs are guided by chemotactic agents such as C3a which is a major chemoattractant factor of serum and inflamed tissue. When PMN cells arrive at the active site of inflammation they may be killed by bacterial toxins or they may remain active with adherence of bacteria to the cell membrane. This is followed by an ingestion process called phagocytosis (Figure 1.1). Phagocytosis is a major defence mechanism by which the immune system protects the body against bacterial infection. Calcium and magnesium ions are required for optimal phagocytosis and anaerobic glycolysis is the energy source. The neutrophil has a recognition site for the organisms on the surface. Virulent organisms are less readily recognised and engulfed. They generally require to be coated with a phagocytosis- promoting opsonin like specific antibody and or C3b for which the cells have receptors on their surface.

IgG binds to bacteria with the Fab end following interaction between the Fc portion and Fc receptors on the cells. Interaction between IgG and Fc receptors opens calcium dependent channels that may be important in regulation of phagocytosis and post-phagocytic events. The PMNs membrane bulges around the particles (bacteria) with formation of a phagocytic vacuole (phagosome). The cytoplasmic granules release their contents into the phagosome. These granules contribute to bacterial killing and lysis. The important event in relation to the killing system is a brief burst of respiratory activity that accompanies phagocytosis with consumption of oxygen. In this process, a variety of products of reduced oxygen are produced like superoxide O_2, hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO). Hydroxyl radicals (HO) is the strongest oxidant known.

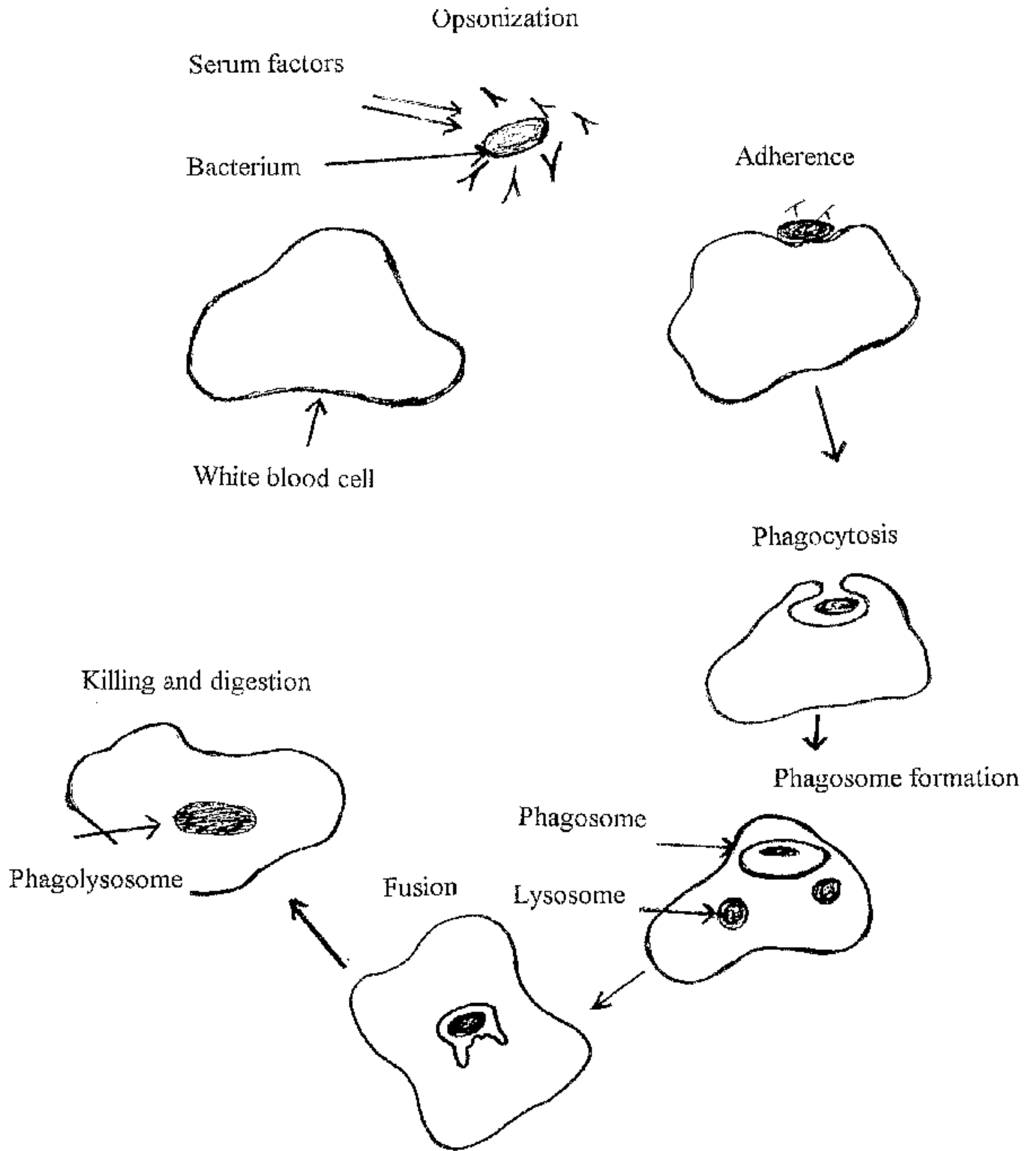

Figure 1.1 The phagocytic process

iii. Impairment of cellular immunity in neonates

The high incidence of bacterial sepsis in the new-born appears to be multifactorial. One of the most important deficits in the neonatal immune system is the quantitative deficiency in the myeloid and phagocytic system[46]. Additionally despite normally circulating numbers of mature affected phagocytes, the presence of serious (in vitro) neutrophil abnormalities may still exist and predisposes the neonate to an impaired immune response during overwhelming bacterial sepsis[45].

A. Neonatal myeloid progenitor deficiency

Christensen et al.[47] have demonstrated significant differences in the neonatal rat myeloid progenitor system compared to its adult counter part. Significantly the neonatal rat has reduced myeloid progenitor cells (CFU-GM) to 10% - 20%. By 4 weeks of age there is a developmental maturation of myeloid progenitor cells to approximately normal adult levels. Additionally it appear that neonatal rat myeloid progenitor cells (CFU-GM) are proliferating at a much higher rate than in adult animals. At birth and through the first week of life the neonatal rat myeloid progenitor cells proliferate at approximately 75% - 80% of maximal capacity compared to 25% rate in the adult rat. Similarly Christensen reported that CFU-GM proliferation rate of premature humans is also near maximum capacity. In the neonatal rat it takes 6 weeks of developmental maturation before myeloid progenitor pools proliferate at a low normal adult level of 25%.

During experimental sepsis adult animals increase their myeloid progenitor pool by almost 100% compared to neonatal rats who decrease their CFU-GM by almost 50%. While during experimental bacterial sepsis in the neonatal rats, they fail to increase their already high myeloid progenitor proliferation rate[48] compared to the adult animals, but in fact they decrease their CFU-GM.

After inoculation with GBS the new-born rat demonstrates substantially different neutrophil kinetics compared to those found in adult animals. Adult animals increase their circulating PMNs count by increasing their stem cell pool two to three times and increasing their stem cell proliferation rate by a similar amount. In contrast neonatal animals under the same conditions fail to increase their stem cell pool (CFU-GM) and may in fact decrease their pool, and also fail to increase their stem cell proliferation rate. This development contributes to reduced circulatory mature neutrophils and increased mortality during sepsis.

B. Neonatal neutrophil storage pool

The neonatal storage pool (bone marrow myeloblast, neutrophil, band cells, metamyelocytes) is severely reduced compared to adult animals[49]. At birth the new-born marrow neutrophil storage pool is approximately 25% that of normal adult animals levels and requires about 4 weeks of development maturation to reach adult levels[50]. Experimental GBS sepsis significantly alters the neutrophil storage pool in the new-born bone marrow compared with adult animals. Neonatal rats deplete their already reduced neutrophil storage pool by almost 80% from previous existing levels compared to a decrease of 33% in adult rats[48].Furthermore, during bacterial sepsis the daily turnover of neutrophils increases dramatically as the half life of the circulating neutrophil decreases. In adult animals and the human being the daily turnover of neutrophils increases during infection from 1.6×10^9 PMN/Kg/day to approximately 5×10^9 PMN/Kg/day. This marked increase in the daily turnover of neutrophils during overwhelming bacterial infection leads to depletion of mature neutrophil storage pool cells. Therefore an adult animal can consume its entire neutrophil storage pool in one day during bacterial sepsis. Since neonates already have a compromised neutrophil storage pool, this increase in neutrophil utilisation contributes to the exhaustion of neutrophil reserves during sepsis and subsequently increases their mortality.

Peripheral neutropoenia is a hallmark finding in overwhelming bacterial sepsis in the new-born and is associated with a poor prognosis. The marked decrease in CFU - GM in the neonate, a near maximum proliferative capacity of myeloid progenitor cells, an accelerated egress and release of neutrophil storage pool cells from the bone marrow reserve and accelerated neutrophil utilisation during infection, all predispose the neonate to a significant neutropoenia. The incidence of severe neutrophils storage pool depletion (<7%) associated with neonatal sepsis has varied in different series and ranges from 15% to 62%[51]. The true incidence of severe neutrophil storage pool depletion associated with neonatal neutropoenia during bacterial sepsis is probably under 15%[52,53]. In the study of Engle et al.[54] it was 6%. This observation may not be the only indicator of a poor prognosis.

C. Physiological function of neutrophils in neonates

Over the years a number of investigators have demonstrated significant in vitro function abnormalities in PMNs derived from neonates. These abnormalities have been found to be even more pronounced during neonatal sepsis or stress. A variety of these abnormalities have been summarised recently by Hill et al.[55]. Specific defects in the neonatal PMN include a decreased deformability, chemotaxis, phagocytosis, C4b receptors expression, adherence, bacterial killing and altered oxidative metabolism[56]. Socchi et al.[57] examine the postnatal maturation of PMNs chemotactic ability after very premature birth and they found a delay in maturation of PMNs chemotaxis in premature babies born before 34 weeks compared with those born between 34-36 weeks. Leonard Eisenfield Peter et al.[58] noted PMN chemotaxis in healthy term neonates was significantly impaired during the first week of life and increased to adult level by two weeks of life.

Miller et al.[59] demonstrated impaired chemotaxis of neonatal neutrophils in response to various bacterial organisms and antigen antibody complexes. Additionally

oxidative metabolic abnormalities have been demonstrated in the PMNs of new-borns. Ambruso et al.[60] reported a decrease in the generation of the hydroxyl radicals of PMN in contrast with normal to increased amounts of superoxide generation of PMNs in the neonates. Bruce et al.[61] have recently reported impaired surface membrane expression of C3b1 receptors in neonatal neutrophils which have been implicated in the abnormal adherence of neonatal PMNs. The adherence of PMN cells is also impaired during the first pos-natal week.

A summary of the quantitative and qualitative myeloid deficiencies observed in the neonatal infant is given below:

A. Quantitative changes

1. Decreased bone marrow myeloid progenitor.
2. Decreased bone marrow myeloid proliferation.
3. Decreased bone marrow neutrophil storage pool
4. Decreased neutrophil count in septic infants..
5. Decreased myeloid response to sepsis.

B. Qualitative changes

1. Decreased deformability and chemotaxis.
2. Decreased adherence and C3b1 expression.
3. Decreased opsonisation and phagocytosis.
4. Decreased bactericidal activity.
5. Decreased oxidative metabolism.

1.5.3 Humoral immunity

a) Immunoglobulin (B lymphocyte cell)

Differentiated lymphocytes subserving antibody production are refered to as B cells (Bone marrow derived cells). B cells originate from a progenitor pool in the bone marrow, characterised by a lack or low concentration of surface immunoglobulin. B lymphocytes synthesise and secrete specific antibody, some of which are incorporated into the cell membrane and serve as receptors for antigen. This surface bound immunoglobulin (Sig) can be identified by a variety of techniques. Approximately 15%-30% of normal peripheral blood lymphocytes have been found to bear Sig. Lymphocytes having surface IgM, IgD, IgG, IgA make up the greatest number of B cells in the peripheral blood in adult. A second marker of B cells is the Fc (crystalline fragment) receptors which binds the Fc portion of the immunoglobulin molecule. B cells can also be shown to have cell surface receptors that interact significantly with two components of complement C3b and C3d.

I. Humoral and opsonic deficiency

Humoral immunity including quantitative immunoglobulin and opsonic activity are markedly impaired in animals and human preterm and term neonates. Preterm infants have a poor response to various antigenic stimuli, a reduced level of gamma globulin at birth due to a reduced maternal supply of immunoglobulin from placental transport and inability to produce their own immunoglobulin. The presence of physiological hypogammaglobulinaemia has been noted by several investigators in preterm and term neonates.

Ballow et al.[4] have reported a reduced level of IgG in preterm infants born between 26 to 31 weeks gestational age, with an increase in circulating

immunoglobulin levels with increasing gestational age, but the levels are still reduced compared with those in term infants. Almost 33% of infants with birth weight of less than 1500 grams develop hypogammaglobulinaemia with IgG levels of less than 200 mg/dl[62]. Neonates have lower concentrations of certain IgG subclasses including IgG2 and IgG4. Neonates therefore are unusually susceptible to pyogenic bacterial infection since most of the antibodies that opsonise capsular polysaccharide antigen of pyogenic bacteria (GBS and E. coli) are contained in these deficient IgG2. Also neonates have low levels of IgA and IgM due to the poor ability of those immunoglobulins to cross the placenta.

Even during overwhelming sepsis the neonate cannot produce type specific antibody. During neonatal sepsis, there appears to be a transient increase in the synthesis of IgM but little production of IgG[63]. This impairment in type specific antibody production, appears to be secondary to defects in the differentiation of B lymphocytes into immunoglobulin secretory plasma cells and T lymphocyte mediated facilitation of antibody synthesis[64,65]. Thus reduced antibody levels in the neonate appear to be secondary to reduced maternal transport of maternally derived antibody and impaired neonatal antibody production secondary to abnormal interaction between B and T lymphocytes in the neonate.

II. Structure of immunoglobulin

The antibody activity of serum and secretion is associated with a heterogeneous group of proteins collectively known as immunoglobulin. Despite their heterogeneity, all antibodies share certain structural similarities. All consist of a subunit composed of four polypeptide chains held together by a disulphide (Figure 1.2) bond. Two have a molecular weight of 53,000 to 75,000 depending on the immunoglobulin class and are known as the heavy (H) chain. The other two have a molecular weight of 22,500 and are known as the light (L) chain. On the basis of their general properties, and the

immunochemical features of their constituent H chains, immunoglobulins are subdivided into five major classes IgG, IgM, IgA, IgE and IgD. Within a given class of heavy chain, subclasses have been distinguished by their antigenic characteristic, for instance there are four subclasses of IgG.

Each H chain is joined to its adjacent L chain by non-covalent forces and a single disulphide bond. The two L-H chain pairs of each immunoglobulin molecule are also linked by non-covalent interaction and by neighbouring disulphide bonds (usually two) between the heavy chains. When 7s gamma globulin is treated with papain in the presence of cysteine, three 3.5s fragments will result. Two of these are identical , consisting of a light chain and the amino-terminal of the heavy chain (Fc fragment of heavy chain). These are referred to as the Fab (antigen binding) fragment. The third piece is known as the Fc (crystalline) fragment. It contains the carboxy-terminal half of both H chains (most of the carbohydrate and the antigen determing class of specificity). The Fc piece mediates fixation of immunoglobulin molecule to cells (skin, mast cells, lymphocyte and macrophage), placental transfers and complement fixation.

1. Immunoglobulin G (IgG)

IgG is the major immunoglobulin in man and constitutes about three quarters of the total gamma globulin. The serum concentration varies from 800-1600 mg/dl in adults but the intravascular pool accounts for less than half of the total body IgG. About 55% is found widely distributed within the extravascular space. Also it is present in external secretions and cerebrospinal fluid. Its molecular weight is 155,000 and it is synthesised at a rate of 33 mg/Kg/day. The total body content is in excess of 1g/Kg of body weight. IgG molecule has a half life of about 21 days and thus it is the longest lived immunoglobulin. On the basis of antigenic determinants within heavy

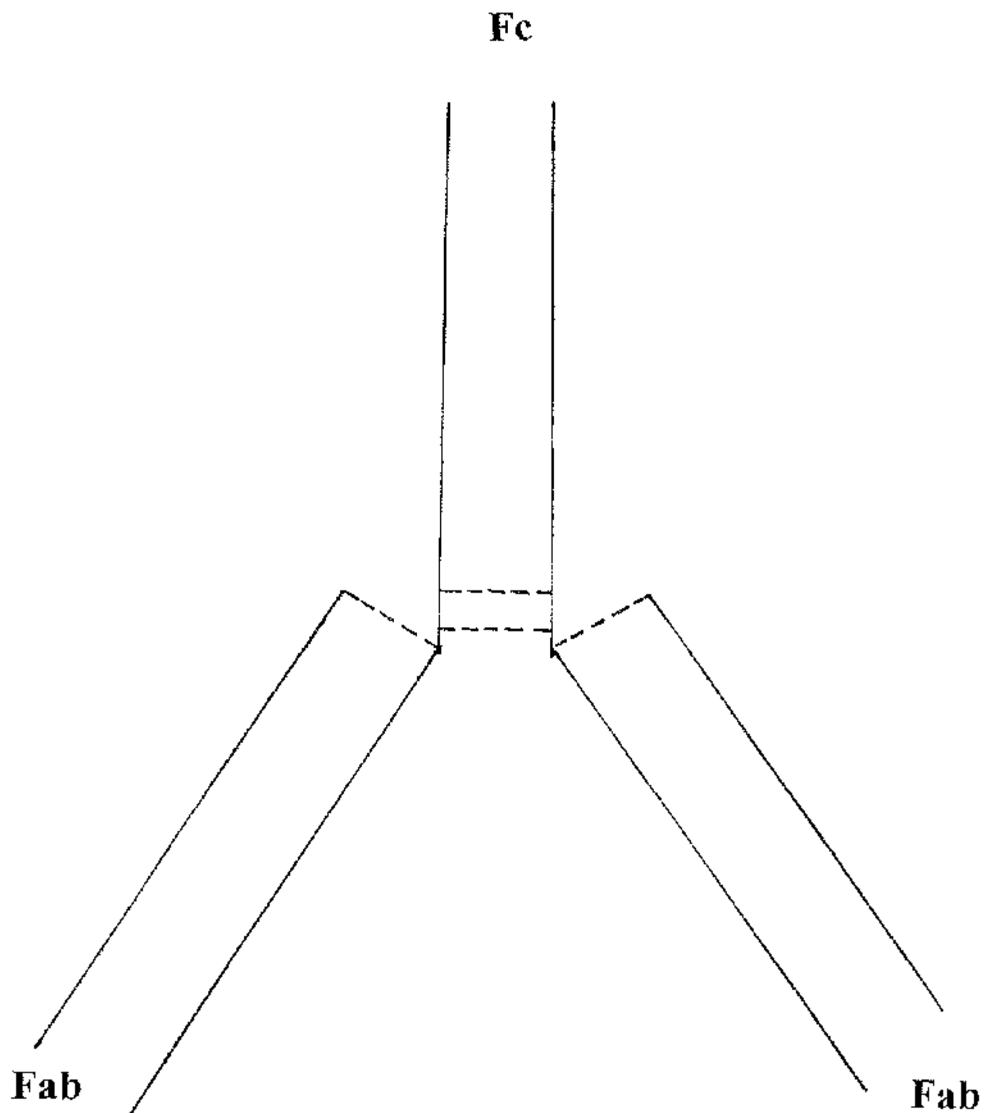

Figure 1.2 The IgG molecule. Antibodies of the IgG class contain four chains of amino acids linked by disulfide bonds. The Fab (fraction antigen binding) and the Fc (fraction crystallizable) portions mediate various immunologic function.

chains of IgG, four isotype subclasses of IgG molecule have been identified in the serum. IgG 1 consists of 66% of total IgG, IgG 2 (23%), IgG3 (7%) and IgG4 (4%)[66].

These antigenic differences are the result of a variation in amino acid sequences of carboxyl-terminal parts of the gamma chain. All four types of molecule are found in any given normal serum. In addition to the antigenic difference that determines immunoglobulin subclass, the protein within each IgG subclass possesses distinctive allotype antigen known as Gm factor. These factors are the products of allelic genes that are associated with the separate ciatrous controlling each immunoglobulin subclass. IgG4 subclass has no recognised Gm marks. Some biological properties of IgG proteins and particularly those mediated by the Fc fragment are distinctively subclass specific.

The complement activation through binding of C1q (the first activated component of complement) is most efficient with IgG1 and IgG3. IgG2 is also active but intact IgG4 is completely inactive[67,68]. The binding of IgG to macrophages and granulocytes Fc receptors is most efficient with IgG1 and IgG3[69,70]. The later reaction is important in opsonisation. Most of the antibodies developing in response to antigen stimulation are IgG. It is the only immunoglobulin selectively transferred across the placenta, thereby giving a measure of protection to the new-born infants[71,72].

Dimitris Sideropoulos et al.[73] studied the transplacental passage of IgG in the last trimester of pregnancy. Intravenous immunoglobulin (IVIG) in combination with antibiotic (amoxicillin and clindamycin phosphate) was given to 27 pregnant women at 27 to 36 weeks with signs of chorioamnionitis who were at risk of preterm delivery. IVIG was given either as a single dose of 12 grams in 12 hours (low IVIG dose) or 24

grams on each of 5 consecutive days (high IVIG dose). 24 patients received the same antibiotic alone (control group). IgG concentration in maternal and umbilical cord sera were measured and the levels of four IgG subclasses were determined.

They found transplacental passage of IVIG was shown to be a function of gestational age and of dose. Up to 32 weeks of gestation, IVIG infusion has no effect on IgG concentration in cord serum. After that time cord serum IgG levels were significantly higher in the high dose group compared with low dose and control group. All four subclasses of IgG and two different antibodies present in the IVIG preparation passed from the mother to the fetus. Thus the infused IgG mimicked the transplacental passage of endogenous IgG. The serum IgG concentration at different gestational ages, in neonates and infants are shown in Table 1.1.-Figure 1.3.

Smith and Hammarstram[74] also demonstrated that the subclasses IgG_1, IgG_2, IgG_3 were transferred from the mother to the fetus. Boyers et al.[75] investigated the transfer of endogenous IgG to GBS type Ia antigen in paired maternal and cord sera. Nearly all GBS can be identified and classified into five distinct serotype (Ia, Ib, Ic, II, III) based on the presence of type-specific capsular polysaccharide and protein antigens present on the cell surface. The presence or absence of type-specific antibody to these antigens appears to correlate with relative risk of developing invasive disease. The ratio of cord/ maternal anti-Ia concentration increased with an increase in gestational age from 0.33 at 28 weeks gestation to 0.49 at 40 weeks of gestation. These kinetics of the passage of anti-Ia antibodies were similar to those observed for non-specific endogenous IgG[71,76]. Physiologically this transfer of IgG antibody is mediated by an active transport mechanism[77] and appears to be initiated by receptors at the syncytiotrophoblast membrane that binds the Fc portion of IgG molecule.

Biological properties of IgG

Most antibodies developing in the secondary response to antigen stimulation are of the IgG type. Some biological properties of IgG are distinctly subclass specific. Complement activation is most efficient with IgG1 and IgG3. IgG2 is also active but intact IgG4 protein is completely inactive.

The binding to macrophages and granulocyte Fc receptors is most efficient with IgG1 and IgG3. The latter reaction is important in opsonization. IgG3 has shown a great tendency to aggregation, which is probably involved in its affinity for C1q. In addition, IgG3 is selectively retained in the sera of a number of patients with generalised hypogammaglobulinaemia. IgG antibodies are efficient in the neutralisation of some toxins and viruses through Fc fragment.

The activation of complement through binding to C1q (the first activated component of complement) depends upon changes in configuration of antibody molecule when they are brought into close apposition during reaction with antigen and it is the Fc fragments of the heavy chain which carries the predominant part of the molecule responsible for complement fixation.

2. Immunoglobulin A (IgA)

The IgA antibodies can be divided into two separate systems of immunoglobulin. One of these provides IgA antibodies for the circulation and the internal secretions such as aqueous humour of the eyes, the cerebrospinal fluid, synovial fluid, amniotic fluid, pleural fluid, and the peritoneal fluids. It is likely that these IgA antibodies are synthesised by nonmucosal lymphoid tissue[78]. The other system of IgA antibodies is found in external secretion such as saliva, tears, bile and colostrum as well as those of the respiratory tract, gastrointestinal tract, seminal vesicle, cervix, and the urinary tract. The IgA of external secretions is not derived

from blood but produced locally by plasma cells situated in proximity to the epithelial mucosa[79]. It is the predominant immunoglobulin in external secretion, although smaller amounts of IgM and IgG may also be found. In the serum 85% of IgA molecules are monomers with a molecular weight of 170,000 and the serum IgA concentration is in the range of 200-300 mg/dl with a half life of 6 days[79]. 42% of IgA is intravascular, and its synthesis is at a rate of 24 mg/Kg/day. It is not transferred across the placenta.

Intrauterine synthesis (Table 1.1) [80] is very minimal and it becomes detectable in the infant by 15 days postnatally in most infants at a level of 1-5 mg/dl. Its concentration increases gradually with age to reach levels approximately 30 mg/dl by 6 months (Figure 1.3).

About 1% of immunoglobulin is secretary IgA[78]. The proportion of secretory IgA increases in a variety of mucosal inflammatory diseases. It has a large molecule with a molecular weight of 390,000.

Biological properties

Secretory IgA is reported to be active against a variety of viruses and bacteria but the mechanism is unknown[78,81]. IgA is incapable of fixing complement or acting as an opsonin. Although IgA does not activate complement by the classical pathway, it can be shown to have activity in the alternative complement pathways[78]. Like other immunoglobulins, secretory IgA is capable of blocking bacterial adherence to mucosal surfaces, thereby preventing colonisation. It may also act as a backing antibody to reaginic reaction on mucosal surfaces and may prevent the absorption of antigenic molecules from the lumen of the gut[78].

Table 1.1. Levels of immunoglobulins (mg/dl) in sera of fetus, new-born and infants by age[80].

Age	IgG	IgA	IgM
-23 weeks	below 100	—	—
-(13-14) weeks	330±31	—	—
-(7-8)weeks	456±107	—	—
-(3-4) weeks	823±135	—	—
At term	1031±200	2±3	11±5
1-3 Months	430±119	21±13	30±11
4-6 Months	427±186	28±18	43±17
7-12 Months	661±219	37±18	54±23
13-24 Months	762±209	50±24	58±23
25-36 Months	892±183	71±37	61±19

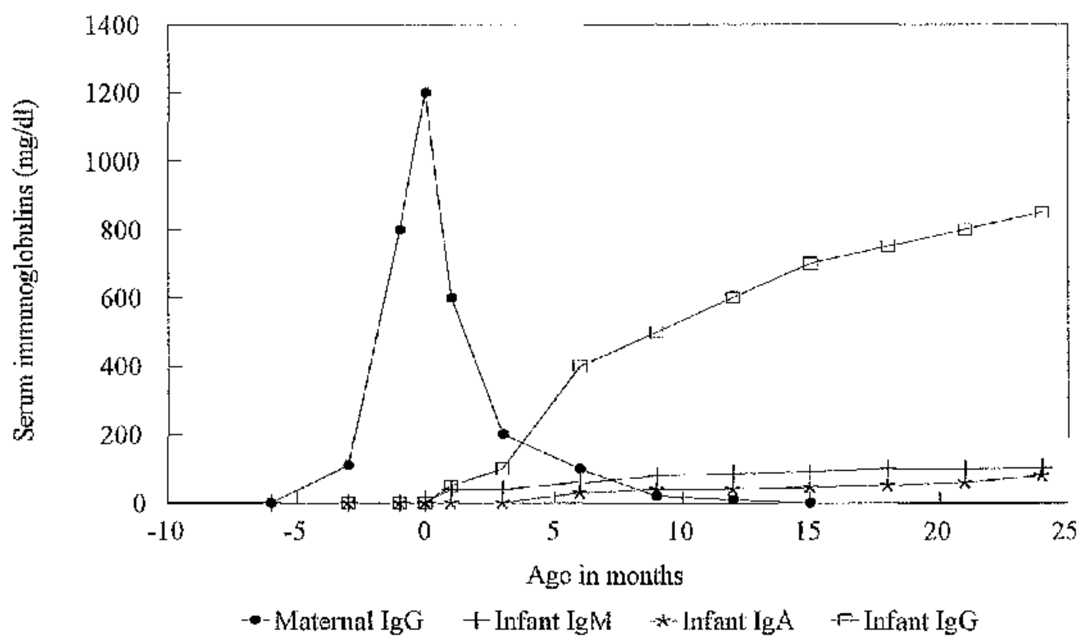

Figure 1.3 Development of immune globulin (mean levels of immune globulin in mg/dl) (Richard V et al. Immunology in the paediatricians office. Clinical Immunology 41;4:691-713,1994)

3. Immunoglobulin M (IgM)

IgM antibodies have a molecular weight of 850,000. Because of their size IgM molecules are refererd to as macroglobulins. Their rate of synthesis is 6.7 mg/Kg/day which is only 1/20 that of IgG whereas their fractional catabolic rate is two to three times higher than that of IgG[82]. This accounts for a relatively short half life of about 5 days and low serum levels of 80-300 mg/dl[83]. IgM can be detected in cord blood after 30 weeks of gestation and increases very slowly with gestation, reaching 10 mg/dl in full term infants with an upper limit of normal ($\pm$ SD) of 20 mg/dl (Figure 1.3-Table 1.1). The IgM molecule consists of five subunits, each with two antigen combining sites. There are 10 antigen combining sites per IgM molecule especially if the antigen is relatively small. 75% of IgM is intravascular. IgM is present in external secretions but not in the cerebrospinal fluid.

Biological properties of IgM

Macroglobulins (IgM) are restricted predominantly to the intravascular pool[83]. Little if any IgM crosses the placental barrier and that present at birth is predominantly of fetal origin. Detectable levels of IgM may be synthesised by the human fetus as early as the twentieth week of gestation[84], but high levels of IgM at birth are usually an indication of intrauterine infection. Specific receptor sites on macrophages for the Fc region of IgM have been identified in animal systems[85]. Such receptors may play a critical role in the phagocytosis of immune complexes. IgM antibodies are the first to be produced in a primary immune response and to be replaced subsequently by IgG antibody. In addition however certain types of antibody response remain predominantly IgM including those against lipopolysaccharide antigen.

4. Immunoglobulin E (IgE)

Reagenic antibodies which mediate acute and sometimes life threatening allergic reactions in atopic patients belong to this distinct immunoglobulin group. It has a molecular weight of 200,000. Their survival in the serum is shorter than that of any other immunoglobulin (half life is 2.4 days). Serum concentration is in the range of 0.01-0.07 mg/dl with a mean of 0.03 mg/dl[86,87]. Higher concentrations may be found in the serum of patients suffering from asthma, hay fever, eczema and helminthic infestation. 50% of IgE is intravascular and is synthesised at a rate of 0.02 mg/Kg/day. It is present in external secretions, but not in cerebrospinal fluid. IgE forming plasma cells are most frequent in the respiratory, gastric and intestinal mucosa and in the regional lymph nodes but few are found in the plasma and other lymph nodes. IgE like IgA is classified as a secretory immunoglobulin.

Biological properties

IgE antibodies are capable of sensitising basophil and mast cells. The Fc portion of the IgE molecule fits into specific receptor sites in the cell surface[87]. When bivalent or multivalent antigens bind to at least two adjacent molecules, the mast cell or basophil cell is triggered to de-granulate, thereby releasing vasoactive substances especially histamine and slow reacting substances. These are responsible for clinical manifestations such as wheel and flare reactions, bronchospasm, small vessel dilatation and shock. IgE may also play a part in normal body defence mechanisms, and has a property of complement fixation through classical and alternative pathways.

5. Immunoglobulin D (IgD)

IgD is found in low concentration in normal serum 0.3 - 40 mg/dl[87]. It has a molecular weight of 180,000, and is synthesised at a rate of 0.4 mg/Kg/day, its fractional catabolic rate (% per day) is 37 and it has a half life of 2.8 days[88]. It is confined largely to the intravascular space (75%) and it does not cross the placental

barrier. IgD has been shown to be a major surface immunoglobulin of peripheral blood lymphocytes[89], and it activates complement by classical and alternative pathways.

b) Fibronectin

Fibronectin is a multi-functional high molecular weight glycoprotein that serves to facilitate cell-to-cell and cell-to-substratum adhesion. It exists in soluble and insoluble forms. Soluble forms are found in interstitial fluid while insoluble ones are present in most extra-cellular materials and are widely distributed in connective tissue throughout the body. Fibronectin displays individual binding sites for some molecules such as actin, complement C1q, serum amyloid P, ganglioside and has multiple binding sites for other compounds[90], such as heparin, fibrin and immunoglobulin G. Multiple species of bacteria (gram positive and gram negative) including potential neonatal pathogens such as staphylococci and streptococci also bind to fibronectin. Human adult plasma fibronectin concentration varies from 260 to 450 ug/ml. Women have a slightly lower level.

The circulating plasma fibronectin concentration in human fetal cord plasma is 120 ug/ml versus 220 ug/ml in the term infants[91]. Premature infants of 30 to 31 weeks gestation have a plasma concentration of 152 ± 34 ug/ml, which is significantly less than in the term infant[91]. The fractional synthetic rate (percentage of plasma pool produced daily) of plasma fibronectin varies from 9% to 31% in infants born at 26 to 32 weeks gestation when measured before 12 days of age[92]. In adult humans it is 35% with a circulating half life of 47 hours[93]. A significant correlation exists between the fractional synthetic rate and gestational age. Youngest infants have the lowest synthetic rate and the longest measured plasma half life is 7.5 days. Thus lower plasma levels may be related to decreased hepatic synthesis of this glycoprotein.

Plasma fibronectin decreases in new-born infants and adult subjects during bacterial infection[94]. This may be due to a decreased synthetic rate or redistribution of fibronectin from intravascular to extra- vascular compartment or due to an increased consumption of fibronectin in response to increased clearance of products of the inflammatory response.

Biological properties

Fibronectin plays an important role in directing cell migration, proliferation and differentiation. It is therefore essential during embryonic development. These glycoproteins perform additional integral functions during homeostasis, immunological clearance of injured tissue and antibody coated micro-organisms. It plays a key part in maintenance of vascular integrity. In certain pathophysiological conditions such as sepsis, multiple organ dysfunction, thrombosis, cancer, arthritis and fibrosis, the normal structure, physiology and function of fibronectin may be altered and in many cases these changes have been implicated as contributing factors to the underlying disease. Fibronectin also plays an important part in defence against infection by mediating macrophage particulate endocytosis[95] and polymorphonuclear leukocyte phagocytosis[96].

It acts as a potent chemoattractant for monocytes and neutrophils in the presence of hyaluronic acid. It also enhances secretion of interleukin-1, and the interaction between phagocytes and the opsonised bacteria. It increases bactericidal activity of macrophages by increasing the affinity, distribution and activity of phagocyte cell surface immunoglobulin (Fc) and complement C1, C3 receptors. In summary fibronectin clearly serves to enhance the function of phagocytes as they react to defend the host from local or systemic microbial invasion or to remove the debris of cellular material at the site of tissue injury.

Potential adverse effects of a decreased concentration during an infective episode include delayed clearance of infection due to decreased opsonisation, impaired neutrophil migration, phagocytosis, localisation to the site of infection and a decreased rate of clearance of products of bacteriolysis. In addition its deficiency may contribute to the development of capillary leak, shock, formation of pulmonary oedema and disseminated intravascular coagulation. Some workers have reported an improvement in immune function after fibronectin transfusion in adults with major illness or trauma[97]. Jacobs et al.[98] have reported that neonatal cord blood monocytes ingest greater numbers of GBS in the presence of fibronectin and intravenous immunoglobulin. GBS ingestion by neonatal cord blood monocytes is less effective when organisms are pre-inoculated with type specific antibody alone, fibronectin plus adult serum, or serum free medium alone.

c) Complement

The complement system is composed of a series of glycoproteins that circulate in the blood and participate in many antibacterial mechanisms, such as opsonisation and bacteriolysis of many pathogenic organisms[99]. There is little placental passage of complement protein which has been identified in fetal tissue by six weeks gestation. Fetal synthesis of C3, C4, C5 by multiple tissues starts between the 8th and 11th week of gestation. Complement synthesis preceeds immunoglobulin synthesis in fetal tissue, suggesting that complement is a more primitive defence mechanism. The first component of complement which is essential for recognition of the tertiary structure alterations of IgG and IgM is produced by the spleen early in fetal life and also by tissue macrophages which are increased through gestation. The fetal serum complement concentration correlates directly with gestation age. In adults, hepatocytes appear to be the principal cells that synthesise complement. Macrophages and fibroblasts also may produce certain complement proteins. In the serum of neonates, the concentration of most complement components is approximately 50%

of that observed in adult serum. For such components, as C8 and C9, the concentrations in neonatal serum are only 10% to 25% of the concentrations observed in adult serum. The serum C3 is much greater than any other component. Compared with the normal adult serum C3 level of 150 to 500 mg/dl, normal cord C3 level is 110 mg/dl. There is a rapid increase in complement levels postnatally, so that by 3 to 6 months of age serum levels are equal to those in adults.

Serum factors necessary for activation of complement are also decreased in the neonate. Factor B (an acute phase protein which acts as one of the humoral factors) is present in the serum of the full term neonate at levels of approximately 50% of maternal serum, and is much decreased in preterm infants in which there is a positive correlation between serum level and gestational age. Factor B is essential for activity of the alternate complement system. Complement is required for the activity of many circulating antibodies and it has been demonstrated that deficiency or consumption of this system contributes to enhanced neonatal susceptibility to infection. The complement system has multiple functions in host defences against invading microbes such as recognition, chemotaxis and opsonization. C1 is a complex molecule consisting of three proteins C1q, C1r and C1s. The C1q subunit is the recognition unit of complement that bears the combining site for the Fc fragment of immunoglobulin and initiates a cascade by reacting with IgG or IgM antibodies that have combined with antigen or have been aggregated by other means. The reaction requires either two IgG molecules that have reacted with antigen in critical proximity to each other or two subunits of a single IgM molecule. Only IgG1, IgG2 and IgG3 antibodies are capable of activating complement. IgG4 does not have this property.

1.6 Breast milk

Human milk is a recommended source of feeding. This recommendation arises from its nutritional benefits, contribution to host defence, and gastrointestinal trophic aspects as well as psychological benefits. The most important aspect of human breast milk's benefit as a natural broad-spectrum, anti-infectious agents was based on the presence of secretory immunoglobulins in colostrum and milk, the presence of cells that could participate in host defences (macrophages, PMN, lymphocyte), complement components, interferon, the presence of microbial growth factors that might allow selective growth of nonpathogenic bacteria, and the presence of secretory molecules that have antibacterial activities (lactoperoxidase, lactoferrin, lysozyme).

All of these factors may affect the host defence of the LBW infants[100,101]. The quantity of host defence factors per unit volume in premature milk is greater than that in term milk[101]. Narayanan[102] reported a lower incidence of infections in LBW infants fed their mother's milk during the day time compared with similar infants fed formula only. Another study[103] shows fewer LBW infants fed human milk developed NEC than similar infants fed formula exclusively.

The predominant human breast milk immunoglobulin is secretory IgA, which is synthesised and stored in the breast, reaches levels as high as 5 mg/ml in colostrum and decreases to 1 mg/ml in mature milk[104]. Secretory IgA acts on the intestinal mucosal surface, resisting breakdown by gastrointestinal fluid, and blocks adhesion of potential pathogens. Levels of secretory IgA against specific pathogens increase in response to maternal exposure to these antigens (environmental specific). This gives the infants access to antibodies directed against specific pathogens and provides protection when his or her immune response is not fully developed[105]. The antibacterial effects of IgA may be amplified through the alternative pathway of

complement activation. Breast milk contains important components of the alternative pathways i.e., factors B and C3.

Mononuclear phagocytic cells are present in large numbers in human milk. All reagents necessary for an effective phagocytic system are present in breast milk. Lymphocytes are also present in human milk at a concentration of about 200/ml, and a sub-population of these lymphocytes produce interferon, macrophage migration inhibition factor and IgA.

Human milk contains lactoferrin and folate-binding proteins that restrict the availability of iron and folate to potentially pathogenic enteric bacteria and fungi. Milk lactoferrin is bacteriostatic[106] rather than bacteriocidal in vitro. It has a strong bacteriostatic effect on E. coli and staphylococci. The highest levels of lactoferrin are found in colostrum and it persists in human milk throughout the first year.

Lysosome provides a non specific protective factor. It is though to influence the flora of the intestinal tract, possibly by killing pathogens through cell wall lysis. Breast milk has a specific factor that encourages the growth of lactobacillus bifidus, which inhibits certain pathogenic bacteria such as Staphylococcus aureus, shigella and protozoa. This growth factor is stable even when milk is frozen or heated. By one month of age, a breast fed infant's bifidobacterium level is 10 times that of a formula fed infant's, the gram negative population being kept low by lysosome and lactoferrin[105].

1.7 Immunoglobulin preparations

Early human immunoglobulin was adminstered by an intramuscular route but its use was limited by painful injections, development of a sterile abscess at the injection

site, local nerve damage or tissue necrosis. The efficacy of these preparations was reduced by low dose, slow absorption, delayed serum peak, local degradation of IgG at the site of injection and spontaneous activation of complement[107].

Intravenous injection of early human IgG preparations was complicated by severe allergic reactions, possibly due to activation of complement by IgG aggregation, which limited its use. Further purification of human immunoglobulin permits the administration of greater amount of antibody with limited anaphylactic reaction.

Currently, there are multiple preparations of human immunoglobulin which have been proven to be safe in use, and may reduce the incidence of bacterial infection among patients with defective immune defence mechanisms. IVIG have been proposed to be effective for the treatment or prevention of bacterial infection among high risk patients with congenital or acquired immune-deficiency states. Currently, there are three different types of immunoglobulins which acts as an effective source of antibody.

Standard IVIG is obtained from pooled human plasma and contains antibodies reflecting those in the age-matched general population and therefore may have deficiencies in antibody against some unusual micro-organisms.

Hyperimmune globulin is prepared by immunising humans against the whole bacteria or a bacterial antigen or obtained from patients known to have a high titer of antibody against a particular pathogen. Passive immunity obtained from these preparations is directed against the specific infection and may include antibody to GBS, varicilla-zoster virus or hepatitis B virus.

Monoclonal antibodies are the most specific immunoglobulin preparation available and are produced by mouse-myeloma cell lines, or by human hybridoma cells, resulting in a chimeric antibody of human Fc and mouse Fab immunoglobulin. Mouse monoclonal antibody therapy has been limited by the development of human antimouse antibody formation.

The most available preparation used for prevention or treatment of bacterial sepsis was standard immunoglobulin. Pooled IVIG obtained from healthy adult donors may not contain functional antibodies to age specific pathogens such as group B streptococci and Escherichia coli and this may result in lot to lot variation in effectiveness. Certain IVIG preparations may have lower titers of functional antibody as determined by functional assays (opsonisation) when compared to non functional assays such as ELISA.

Highly purified human immunoglobulin has been available since the last decade. Sandoglobulin is one of the most widely used in Europe. It is a Swiss Red Cross pooled polyvalent IgG concentrate produced by alcohol cryoprecipitation and mild acidification at pH 4 from plasma known to be negative for hepatitis B antigen and antibodies to HIV.

Gammagard intraglobulin and Venogamma polyvalent are also IgG concentrates made by ethanol fractionation of plasma. An IgM enriched immunoglobulin has recently been developed.

1.8 Preparation characteristics and pharmacokinetics of IVIG in low birth weight infants

Current preparations of intravenous immunoglobulins are all derived from large pools of plasma obtained from thousands of donors. The preparations are initially

fractionated by a Cohn cold ethanol fractionation process. These processes render the products free from viruses such as hepatitis B and HIV which have not been transmitted by current IVIG preparations[108]. Current preparations have proved extremely safe and effective in the management of antibody deficiency syndromes. This acceptability has led to use of high doses of IVIG in a variety of other conditions including Kawasaki syndrome and immuno-thrombocytopenic purpura. In most commercial IVIG preparations, the IgG is further purified by ion exchange chromatography. Most preparations are available as lyophilised powders that are stabilised by the addition of sugar such as maltose or sucrose. IVIG is generally administered as 5% or 6% solution, although in some instances 10% may be administered.

Although the large donor pools should provide antibodies against a wide range of pathogens, specific antibody titres are not standardised. Titres against those pathogens that particularly cause problems in the neonate, such as type III GBS and cytomegalovirus, show considerable variation not only between the preparations of different manufacturers but also between different lots from the same manufacturer[109]. An in vitro[110] study of one IVIG preparation showed that IVIG can increase the opsonophagocytic activity of cord serum for Staphylococcus epidermidis which is an increasingly important neonatal pathogen. Although most preparations contain an amount of IgA, which may cause an anaphylactic reaction in sensitised adults, this has not been reported in neonates, probably because IgA does not cross the placenta.

In the animal studies, the administration of IVIG accelerated the egress of neutrophils from the bone marrow, enhanced the migration of neutrophil to the site of infection, hastened recovery from the infection and thereby prevented neutropenia and depletion of the neutrophil pool.

Several investigators have studied the pharmacokinetics of IVIG in neonates. It fits into a two phase disappearance curve. The first alfa phase is short, beginning immediately after the infusion is administered, lasts 1 to 4 days and may represent redistribution of IgG between intravascular and extravascular spaces[111]. The serum IgG levels decline by 20% to 30% of post-infusion peak levels by 24 hours after an infusion and up to 50% 3 to 4 days post-infusion.

Noya and colleagues[112,113] observed a 40% decline in serum IgG levels within one day of IVIG infusion among very low birth weight infants. This is in part due to extravascular distribution and clearance of denatured or aggregated IgG.

The second component is the beta half life, representing the catabolism of IVIG. This is linear and may be a better determinant of the required dose and dosing interval to maintain therapeutic serum IgG levels. The kinetics of IVIG in neonates as measured by the half life of serum IgG level, are quite variable and difficult to predict[114,115]. Serum half life is altered by gestational age, postnatal age, the presence or absence of infection, the level of total serum IgG and may even be altered by antibody- pathogen specificity[116]. In new-born infants additional factors affecting pharmacokinetics include the dose of IVIG used, metabolic rate, functional activity of the IVIG and the size of the extravascular space. The combination of all of these factors leads to a wide range of serum half lives which varies from 13 to 31 days[111,112,114].

More recent studies[113,114] in babies with a birth weight less than 1000 grams showed that the dose of IVIG infused into these infants may be considerably larger than in normal adults and also may be extremely variable. Even relatively large doses of IVIG may not give the expected increment in serum IgG level. For example the administration of 500 mg/Kg body weight (a relatively large adult dose) is unlikely to

give satisfactory increments in the serum IgG levels in VLBW infants, and may not sustain satisfactory IgG levels for 14 days. In addition the half life of IVIG given soon after birth to VLBW infants may be shortened although this appears to normalise rapidly with post-gestational age[113]. For these reasons initial infusions of 700 mg/Kg are recommended for babies with birth weight under 1000 grams and initial infusions of 500 mg/Kg are for babies weighing 1000 grams to 1500 grams.

For optimal use of IVIG, dosage should be calculated on an individual basis. Serum IgG should be checked 48 to 72 hours after the initial infusion, allowing for the IVIG to equilibrate between the extravascular and intravascular compartments and again 14 days later.

1.9 Side effects of IVIG

In adults and older children many adverse reactions have been reported in association with the administration of IVIG, including volume overload, hypertension, hypotension, headache, flushing, fever, chest pain, bronchospasm, anaphylaxis, laryngeal oedema, haemolysis and asymptomatic hepatitis C[117]. The adverse effects were common in early experimental IVIG preparations and may have been due to aggregates of IgG that could activate complement and cause release of other inflammatory mediators by cross-linked Fc receptors of phagocytic cells, resulting in signs and symptoms associated with anaphylactic reaction. Current IVIG could cause similar reactions. The mechanisms are similar but are caused by antigen-antibody complexes that form rapidly when large amounts of antibody come in contact with the antigen load of an established infection[118]. Clinically important haemolytic reactions caused by antibody to blood type antigen appear to be extremely rare with low doses of IVIG therapy. A true hypersensitivity reaction may produce anaphylaxis or milder allergic reactions which may be due to the development of IgE anti-IgA

antibody in patients with the congenital absence of IgA[119]. Stabilisers or preservative may also cause allergic or other toxic reactions. Contamination with infective agents is a fear but it is an extremely rare event. HIV and hepatitis B have not happened after the use of IVIG. However a few reports of non A non B hepatitis (hepatitis C) have been published following the use of IVIG preparations prepared and used in Europe[114] but this has not been reported in the United States. In most centres all donated plasma from which IgG is derived is tested for known infectious agents. Furthermore the process of purifying IgG removes or inactivates many viruses[120,121]. However technical errors in the production of IgG are possible. The administration of IVIG to neonates appears to be safe, since significant adverse reactions were not observed.

High doses of IVIG seem to block the reticuloendothelial system and may suppress immunity due to decreased bacterial clearance and survival[122] in infected animals, decreased adult monocyte and neonatal neutrophil phagocytosis of bacteria in vitro[123]. There have been reports of fatal infection in immuno-compromised adults after IVIG treatment[124]. Neutropoenia in conjunction with reticuloendothelial blockade delays clearance of bacteria such as E. coli from blood when reticuloendothelial blockade by itself has no effect. In a neutropenic infected adult, IVIG infusion has been associated with fulminant fatal candida albicans sepsis[124].

The data from a study by Weisman et al.[122] indicate that neonates may be particularly susceptible to the adverse effects of high doses of IVIG because neutropenia is common during episodes of infection. Neonatal neutrophils are known to have defects in adherence, aggregation, phagocytosis and intracellular killing. This may be further compounded by neutropenia during infective episodes. Although the exact mechanism of the potential reticuloendothelial blockade is unknown, it may be mediated by Fc receptor blockade. The Fc portion of IgG appears to bind to Fc

receptors in the reticuloendothelial cells. IVIG contains organism-specific antibody directed against a wide range of organisms (based on the experience of the donor pool) but only a small portion of the IVIG may be directed against any one specific organism. Large doses of IVIG may result in a relative excess of non-specific IgG that blocks Fc receptors from binding specific IgG. There is delayed bacterial clearance which is seen 24 hours after the high doses of IVIG were given. This immune suppressive effect of high doses of IVIG may be short lived. The study by Weisman et al.[122] speculates that high doses of non-specific IgG may cause a blockade of neutrophils or bacterial receptors necessary for opsonophagocytosis of GBS. Monoclonal antibodies or IVIG preparation with a high concentration of organism-specific antibodies may reduce the dose and the volume of drug required and result in potentially fewer doses or volume related complications.

In addition to the reported side effects of IVIG, other reactions are theoretically possible. Infusion of IgG into neonates with E. coli sepsis could possibly induce complement mediated lysis of the bacteria. Rapid lysis of bacteria may liberate endotoxins into the systemic circulation and thereby increase the likelihood of shock and death[125,126]. Also it is theoretically possible that antibacterial antibodies could mediate tissue injury in the neonatal recipient. Pathogenic bacteria may express capsules that immunologically mimic components of human tissue, thereby impairing recognition of the organism by the host. For instance, from fetal and infant brain tissue, Finne et al.[127] isolated sialic acid which was indistinguishable from the sialic acid comprising the K1 capsule of E. coli. Infused anticapsular antibody might bind not only to bacteria but also to tissue of the neonatal host and thereby mediate a destructive inflammatory reaction. IVIG did not suppress the production of endogenous IgG nor the expression of natural killer cells activity in premature neonates[128].

There were a number of reports of acute deterioration in renal function and pulmonary toxicity following infusion of IVIG in adults. The Scottish National Blood Transfusion Service was notified of 3 cases of adult patients who developed acute renal failure soon after infusion of IVIG[129].

A recent review reported 18 cases of increased serum creatinine following IVIG[130], nine of whom were diabetic and seven were over 70 years of age. The cause of renal toxicity of IVIG remains to be determined. However it is known that small changes in plasma viscosity may affect capillary blood flow[131] and high doses of IVIG can result in increased plasma and blood viscosity[132]. Part of this effect is due to the large amount of sugar used as a stabiliser of the IVIG. This may explain why the renal toxicity is generally of short duration and is self limiting.

Studies done on the safety and side effects of IVIG

Chirico et al.[133] studied the safety and effectiveness of IVIG for prophylaxis of infection in 133 high risk neonates. They did not observe any anaphylactic, inflammatory reactions or hepatitis after gamma globulin administration.

Von Muralt et al[134] studied children 2-3 years of age who had received high doses of IVIG during the neonatal period for treatment of sepsis. They found no significant difference in serum IgG and IgM levels, response to vaccination nor to skin tests for cutaneous delayed hypersensitivity and anti IgG antibody levels. These data suggest that IVIG replacement therapy does not cause depression of humoral or cellular immune response or abnormal sensitisation to polycolonal IgG.

The study by Noya et al.[112] study included 20 neonates with very low birth weight (750-1500 grams) who received a single dose of 500 mg or 750 mg/Kg during the first week of life. They found a modest decrease in haemoglobin and haematocrit

value which has been reported in another study[135] and appears to be the result of haemodilution due to a high protein content in the IVIG preparation used. Also they found a decrease in complement level CH 50 after IVIG which was followed by a rebound increase above the preinfusion values in many patients. These changes however were not associated with clinically detectable adverse effects.

Clapp et al.[136] studied the administration of IVIG for the prevention of infection in preterm infants with birth weight of 600-2000 grams in an attempt to maintain a therapeutic serum target level of 700 mg/dl in a double blind controlled trial. 115 patients were included. They observed one patient had one episode of transient tachycardia with a decrease in blood pressure during a single infusion. They did not detect any evidence of haemolysis, nor hepatic nor renal impairment in the IVIG recipient. Also they found the infants in the IVIG and placebo group (albumin) had the same incidence of intraventricular haemorrhage but they both had a higher incidence than the non-assigned group. This finding may be related to the osmolarity and volume of IVIG and albumin (placebo) infusion or may be due to the relatively better overall condition of the non-assigned group. Previous data indicate an association between intraventicular haemorrhage and hypertonic infusion only with rapid infusion[137].

Weismin et al.[138] studied 735 neonates with birth weights of 500 - 2000 grams, gestational age of 34 weeks or less and age of 12 hours or less in a multicentre, double blind controlled trial. The incidence of suspected infusion-related adverse reactions was 0.5% and included hypotension and hypoglycaemia. Gonalez et al.[139] used IVIG in doses of 500-700 mg/Kg which was administered to term and preterm neonates without serious effects. In the study by Kanokaudi-Tsakalidou et al.[140] IVIG was given prophylactically to neonates weighing 1000 to 2000 grams at conventional doses of 500 mg/Kg every 10 days, resulting in maintenance of a

satisfactory serum levels of IgG during the high risk period. Such treatment did not have a suppressive effect on subsequent serum immunoglobulin concentration. Homan et al.[141], administered 500 mg/Kg of IVIG to 10 acutely ill infants with proven or suspected sepsis and to 10 clinically well preterm infants less than 1750 grams birth weight as prophylaxis for infection. There was no incidence of hypersensitivity, inflammatory or toxic reactions as assessed by changes in the physiologic and laboratory parameters studied.

These studies suggest that any short term toxicity of IVIG in neonates is minimal. All studies agreed that IVIG infusion was well tolerated by very low birth weight infants. Non of these studies however addressed potential long term adverse effects.

1.10 Review of studies for use of intravenous immunoglobulin (IVIG) for treatment of neonatal infection

Sidiropoulos[142-144] in was the first investigator to report the use of IVIG to treat established bacterial sepsis in human neonates. The study was randomised, but not blinded and placebo was not given.

Eighty two neonates with suspected sepsis received antibiotics alone or antibiotics plus daily infusion of IVIG (Sandoglobulin) for 6 days. Sepsis was proven in 49% and 37% of the immunoglobulin and placebo treated babies respectively. Gram negative rods accounted for 63% of bacterial infection. Deaths occurred in 10% and 27% of immunoglobulin treated and control groups respectively. Deaths in infected preterm babies (<38 weeks) were 8% in the immunoglobulin recipients and 44% of the control group babies.

However the administration of IVIG was associated with a decreased mortality in neonates weighing less than 2500 gram at birth. This result suggests that the administration of IVIG may enhance survival in the low birth weight septic neonate.

Haque et al.[145], reported a randomised, double blind, placebo controlled study in 60 neonates with suspected early onset sepsis treated with antibiotics for 10 days plus 4 daily infusion of either 250 mg/kg/day of IVIG (5%) Pentaglobulin or 5 ml/kg/day of 10% dextrose. (Pentaglobin is stated to be IgM enriched). Sepsis was proven in 70% of babies in the immunoglobulin group which was not significantly different from 77% incidence in placebo group. A large proportion of infection were gram negative organisms (89%). Death occurred in 5% of immunoglobulin recipients compared to 17% of placebo recipients which was significantly different. This study concluded that IVIG enhanced the survival of the septic neonates.

Friedman et al.[146], utilised IVIG to treat ill neonates who were neutropoenic (WBC <3500) and whose blood contained high titers (≥1:10) of group B streptococcal antigen by latex agglutination. This study was not randomised, blinded or placebo controlled. Twelve neonates with neutropoenia and high titres of GBS antigen received 800 mg/kg/day Sandoglobulin in addition to antibiotic. The dose was repeated daily for a minimum of four doses if neutropnia persisted, compared to 12 patients treated with antibiotics alone. 17% of IVIG recipients died compared to 58% of control patients. This study supports the concept that IVIG may enhance survival in neonates with GBS sepsis, even if it has been administered after the development of neutropenia. In addition this study suggested that IVIG may enhance recovery from neutropenia although the difference was not statistically significant. Another study[147] has also reported enhanced recovery from neutropoenia following 750 mg/Kg of IVIG (Gamimune-N) in neonates with clinical signs of early onset sepsis.

Weisman et al.[138], in a multicentre double blind randomised, placebo controlled trial, enrolled 753 neonates of ≤ 2000 grams birth weight, ≤ 38 weeks gestational age and ≤ 12 hours old. They received a single dose of 500 mg/kg of IVIG (Sandoglobulin) or 5 mg/kg albumin. Early onset sepsis was diagnosed in 4.1% mainly due to GBS and E. coli. Among septic babies death occurred in 29% of placebo group and none in the IVIG treated patient within the first 7 days of life, which was significantly different. There was no significant difference in deaths within the first 56 days of life (including the first 7 days). This study suggests that IVIG may enhance the survival of neonates with early onset bacterial sepsis.

Friedman, Sidiropoulos and Weisman studies suggested that there may be a benefit in giving IVIG but Haque did not find much benefit. These studies had a major design difference (e.g., non blinded investigators, small number of patients, historical controls and method of analysis). In addition the studies differed in their doses of IVIG, source of IVIG, rate and severity of infection, and infecting organisms. Because of these design difference, these cumulative data should be viewed with caution. These data suggest that IVIG in addition to antibiotic therapy given to treat neonatal infection, may be effective.

1.11 Review of studies of uses of intravenous immunoglobulin (IVIG) in prophylaxis of neonatal infection

Early studies on immuno-prophylaxis and therapy in neonates were carried out by administration of intramuscular IgG preparation. The first study using intravenous preparation by Sidiropoulos[143] documented a significant reduction in the mortality of infected preterm infants. The results of this study encourage many clinician to utilise IVIG therapy.

Haque et al.[148], administered 120 mg/kg of Intraglobulin to very low birth weight (<1500 grams) neonates on day one of life (50 neonates) or a similar dose at birth and at eight days of life to another 50 neonates. Another 50 neonates were untreated and served as a control. All neonates were observed for infection during the first 12 days. Proven bacterial sepsis developed in 4%, 4% and 16% of patients who received one dose, two doses and in the placebo group respectively. There was a reduction in the rate of infection from 16% to 4% with no deaths in the treated infants but a 25% mortality rate in infected babies in the control group. Gram negative organisms were isolated from all septic neonates and all septic episodes occurred during the first 76 hours of life. These data suggested a protective effect of IVIG against early onset bacterial sepsis with gram negative organisms.

Chirico et al.[133], administered a 500 mg/kg dose of IVIG (Sandoglobulin) on the first day of life, then weekly for a total of four doses to 43 neonates with birth weight ≤1500 grams and gestational age of 24 to 34 weeks. 40 patients served as control, in this randomised but not blinded study. Five per cent of IVIG recipients developed sepsis and one of the two infected babies died during the three month period of observation. In the placebo group 20% of neonates developed sepsis and 75% of infected neonates died. Gram positive organisms were isolated in 60%. During the study period, IVIG was also administered to premature infants who weighed more than 1500 grams at birth. No protective effect of IVIG was identified in these larger premature neonates.

Chirico's study was the first to suggest a protective role of IVIG in the prevention of late onset nosocomial sepsis in VLBW infants.

Stabile et al.[149], administered 500 mg/kg/day of IVIG (Venogamma) on days 1 , 2 , 3 , 7 , 14 , 21 and 28 days of life to 46 neonates weighing ≤ 1500 grams or ≤ 34

weeks gestation. Forty eight (48) served as controls. Although the study was randomised and controlled, it was not blinded and the controls did not receive placebo. Proven sepsis was observed in 13% of IVIG recipients (three of the five infected babies had meningitis) and in 8% of the controls (one of three infected babies had meningitis). Infection occurred from 2 to 25 days of age. Deaths occurred in 80% and in 33% of IVIG recipient and control group with proved sepsis respectively. The organisms isolated from blood of infected neonates included S. epidermides, S. aureus, Serratia, Klebsiella, Pseudomonas and Listeria. The results of this study were unable to confirm any significant effect of IVIG in the incidence of sepsis in preterm infants. However the number of subjects enrolled in this study may have been insufficient to detect a significant difference in the incidence of sepsis between the two groups.

Bussel et al.[150], studied 126 neonates weighing ≤ 1300 gram at birth. They were included in a randomised double blind controlled study. Sixty five received placebo and 61 received four doses of 1000 mg/dose IVIG (Sandoglobulin) in the first 5 days of life and again on day 15. By 30 days of life, 25% placebo recipients and 15% of IVIG recipients developed proven bacterial sepsis. By 70 days of life, the incidence of sepsis did not differ between the placebo and the IVIG recipients.

The results of this study suggest that prophylactic IVIG may diminish the incidence of nosocomial bacterial sepsis in VLBW neonates but the effect may not be sustained if the IVIG is administered only during the first few days of life.

In a randomised, double blind, placebo-controlled study, Clapp et al.[136] treated fifty six preterm infants with birth weight from 600 to less than 2000 grams with IVIG (Sandoglobulin) in the first 48 hours of life then every two weeks until discharge to maintain serum IgG at 700 mg/dl. Fifty nine neonates with the same birth weight

received a placebo. Another 85 infants who were not enrolled in the study due to parent's refusal, were followed for incidence of infection. The initial dose of 500 mg/kg of IVIG was given to neonates weighing ≤1000 grams and 700 mg/kg to neonates weighing >1000 grams at birth. The subsequent doses of IVIG (200 to 900 mg/kg) were adjusted to maintain the serum IgG concentration above 700 mg/dl.

IVIG recipients did not suffer from any septic episodes while 12% of placebo treated babies and 10% of non randomised patients had at least one episode of sepsis. At the onset of infection the serum IgG concentration was less than 400 mg/dl. The incidence of infection between the three groups was significantly different. Thirty eight per cent (38%) of the septic episodes were due to S.epidermidis, 13% due to S. aureus and 25% were due Candida.

No infants with birth weight greater than 1300 grams developed infection therefore IVIG was not shown to reduce the incidence of infection. IVIG did not affect the incidence of deaths or necrotising enterocolitis.

This finding suggest that when IVIG is administered repeatedly in doses sufficient to achieve a serum concentration ≥ 700 mg/dl, it can reduce the incidence of nosocomially acquired sepsis in VLBW infants.

Baker et al.[151], in a multicentre, randomised, double blind placebo controlled study included 235 neonates with birth weight 500 to 1750 grams who received placebo and 229 who received 500 mg/kg IVIG (Gammagard) between days 3 and 5, after one week and then at 14 day intervals up to 5 doses. Proven bacterial sepsis occurred in 46% of babies in the placebo group and 34% of IVIG recipients. The reason for the unusually high incidence of bacterial sepsis in the control group was not clear. The administration of IVIG was associated with diminished length of

hospitalisation and a reduction in the need for insertion of central venous line. There was no difference in mortality rate between the two groups. The infusion of IVIG did not affect the incidence of sepsis in neonates weighing ≥ 1500 grams at birth, the incidence of necrotizing enterocolitis and the rate of survival.

This large multicentre study suggests that repeated doses of IVIG may reduce the incidence of bacterial sepsis in VLBW infants.

Weisman et al.[152] reported the result of a multicentre, double blind and placebo controlled study designed to assess the efficacy of a single dose of IVIG administered prophylactically within the first 12 hours of life to neonates weighing 500 to 2000 grams at birth. Three hundred and eighty one (381) neonates received placebo and 372 received 500 mg/kg IVIG (Sandoglobulin).

The incidence of bacterial sepsis, death and necrotizing enterocolitis was unaffected by the administration of IVIG. This study suggests that, when IVIG is administered as a single dose on the first day of life, it does not reduce the incidence of nosocomial infection.

Conway et al.[153], administered 200 mg/kg of IVIG (Intraglobulin) within 48 hours of life then every 3 weeks during hospitalisation to 29 neonates with a gestational age less than 30 weeks. 26 neonates served as controls in this randomised but not placebo controlled study. Although the incidence of apparent infection was reduced in the IVIG recipients, the incidence of culture proven sepsis (8 of the 29 neonates) was not significantly different from that of the controls (14 of 26). Therefore the results of this study did not indicate that IVIG diminished the incidence of bacterial sepsis of VLBW neonates.

Magney et al.[154], in a randomised blind and placebo controlled study, administered 500 mg/kg of IVIG (Biotransfusion) or albumin on days 0, 1, 2, 3, 17 and 31 to 235 premature infants born at ≤ 32 weeks gestation and less than 25 hours old with an endotracheal tube ± umbilical catheters on admission. They were observed for infection for 45 days. This dose was reported to maintain IgG levels ≥ 500 mg/dl for at least 45 days. They counted infection before the 5th day as maternal in origin. Nosocomial infection occurred in 38% of IVIG treated babies and in 31% albumin treated babies and were not significantly different. 15.5% of infected infants in the IVIG treated group and 14% of infected infants in the albumin treated group died from septic complications.

Fanaroff (NICHD) study[155] was a multicentre, prospective, randomised, controlled trial. They included infants with birth weights between 501 and 1500 grams. Twelve hundred and four (1204) infants received IVIG, in a dose of 900 mg/Kg to infants with birth weight 501 to 1000 grams and in a dose of 700 mg/Kg to infants with birth weight between 1001 and 1500 grams within 72 hours of birth then every two weeks until they attained a weight of 1800 grams or discharged from hospital. Nosocomial infections were encountered in 17% and 16% of the IVIG and control group respectively. The result of this study failed to demonstrated any reduction in the incidence of nosocomial infection.

Summary: Many studies[133,136,148,150,151] suggest that IVIG may be beneficial in preventing neonatal infection. While other studies[149,152-155] found no benefit for IVIG in prevention of neonatal infection.

The cumulative data of these reports suggest that IVIG in prophylactic use to prevent neonatal infection may not be effective in the current products or regimens.

Chapter 2

2.1 Study population

During the period of the study between September 1993 to August 1994 all infants born at Glasgow Royal Maternity Hospital with gestational age of 32 weeks or less, and weighing 1500 grams or less at birth, who needed intensive life support and whose parents consented to their participation, were included in the study. Intensive life support means those infants needing assisted ventilation, and those who had an umbilical catheter and or peripheral long lines for the first 2 days of life. Infants who did not require intensive life support were excluded from the study on the basis of being clinically well.

From September 1993 to October 1994 ninety two (92) babies with birth weight ≤1500 grams and born at thirty two (32) weeks of gestation or less, were admitted to the neonatal intensive care baby unit at Glasgow Royal Maternity Hospital. Thirty eight (38) were included in the study.

Fifty four (54) infants who were not enrolled in the study were either:

1. Clinically well babies who did not need intensive life support.
2. Babies needing intensive care but whose parents refused to enrol them in the study.

Out of a total of 38 babies, nineteen (19) infants were randomised to the treatment group (IVIG) and nineteen (19) infants to the control group. Two babies from the control group died early and were excluded. Three babies from the treatment group died, two of them died early (on day two and four) and were excluded from the study. The third infant died at the age of thirty four (34) days. He received three IVIG

infusions, and died on the third day of the third infusion. This baby was included for the 14 days period of post infusion for two infusions but not after the14 days post infusion period.

As can be seen in Table 2.1 there was no statistical difference in gestational age, birth weight, mode of delivery, and Apgar score between the two groups. But there was a statistical difference in the administration of steroid to the mothers before delivery. Nine (9) out of seventeen (17) mothers in the treatment group received steroid (52.9%) in comparison to fifteen (15) out of seventeen (17) in the control group (88.3%). Steroid and thyroid releasing hormones were given to the mothers before premature delivery to enhance fetal lung maturation.

All the infants in the two groups developed respiratory distress syndrome and were ventilated. There was no significant difference in its severity, and ventilatory requirements among the two groups. However there was a significant difference in the incidence of air leak ($p < 0.05$ student's t test). Three (3) out of seventeen (17) in the treatment group developed pneumothorax (17.6%) while none of babies in the control group developed air leak. There was no statistical difference in the incidence of patent ductus arteriosus and intraventricular/periventricular haemorrhage ($p > 0.05$).

Medical management was dictated by the attending physician. Additional therapies include administration of other blood products and pharmacological agents.

In the group of babies receiving IVIG, 14 out of 17 babies (82.3%) received at least one blood transfusion and 8 out of 17 babies (47%) received FFP. In comparison, in the control group 15 out of 17 babies (88.2%) received at least one blood transfusion and 10 out of 17 babies (58.8%) received FFP. The mean numbers

of transfusions was similar in the two groups. Two babies in the IVIG group (11%) received platelets and no patient in the control group received platelets. These results indicated no significant difference in blood and fresh frozen plasma (FFP) transfusion before the infusion and during the 14 days period post infusion between the IVIG group and control group. These babies received blood and FFP as part of their intensive care management.

2.2 Methods

On admission all infants with gestational age of less than 35 weeks were given 500 micrograms of vitamin K intramuscularly as a prophylaxis for a bleeding diathesis of the newborn. Also all infants with gestational age of less than 32 weeks were given vitamin E 25 mg intramuscularly daily for three days from birth then 30 mg orally as prophylaxis against retinopathy of prematurity and intraventricular haemorrhage. Preterm babies with idiopathic respiratory distress syndrome needed intubation, assisted ventilation, umbilical arterial catheterisation and/or umbilical venous catheterisation and cover with antibiotics, Benzyl Penicillin and Netilmicin. This was the standard therapy used in the neonatal unit.

The parents were interviewed, the aim and the nature of the study were fully explained and informed written consent obtained from the parent. The study protocol was approved by the Ethics Committee of the Glasgow Royal Infirmary. As soon as parental consent was obtained, the baby was able to enter into the study. Randomisation was done by the hospital pharmacist and the baby was entered into one of the study groups. One group received IVIG at a dose of 500 mg/kg body weight to babies weighing 1001 to 1500 grams and 700 mg/kg body weight to babies weighing 1000 grams or less within 2 days of birth. The other group (control) received an equivalent volume of 5% dextrose on a double blind basis. These doses

Table 2.1 Clinical data on treatment and control groups.

	IVIG group (n = 17)	Control group (n = 17)
Sex M/F	11/6	11/6
Gestation age	27.7 ± 2.6 weeks	28 ± 2.23 weeks
Birth weight	1041 ± 257 grams	1065.9 ± 208 grams
Mode of delivery		
S.V.D.(%)	7/17 (41%)	5/17 (30%)
Forceps (%)	—	1/17 (6%)
LUSCS	10/17 (59%)	11/17 (65%)
Birth asphyxia	2/17 (11.7%)	1/17 (6%)
Apgar score at 5 min.	8.23 ± 2.38	8.58 ± 1.90
PROM	4/17 (23.5%)	4/17 (24%)
Steroid to mother	9/17 (52.9%)	15/17 (88%)
Need of surfactant (%)	12/17 (70.6%)	14/17 (82%)
Ventilation (%)	17/17 (100%)	17/17 (100%)
Duration of ventilation	11.7 ± 14.7 days	10.3 ± 12
PDA	7/17 (41%)	4/17 (24%)
Air leak (%)	2/17 (17.6%)	—
IVH/PVH (%)	7/17 (41%)	10/17 (59%)
Duration of antibiotics (days)	17.2 ± 11.6	14.3 ± 1.2
Umbilical A. catheter (%)	13/17 (76.5%)	10/17 (59%)
Duration (days)	5.7 ± 2,6	5.5 ± 2.6
Umbilical V. catheter (%)	10/17 (58.8%)	9/17 (52%)
Duration (days)	3.5 ± 1.5	3.2 ± 1.5
Long line	12/17 (70.5%)	12/17 (71%)
Duration (days)	9.6 ± 4.8	10.6 ± 6.6
Days in I.T.U.	30.9 ± 24.8	31.2 ± 23.7
Days in Hospital	59.8 ± 36.6	56.9 ± 32.5

Mean value ± S.D.

were repeated at fortnightly intervals throughout the duration of the baby's stay in intensive care and needed intensive care support.

5% dextrose was chosen on the basis of it being similar in appearance and of the same concentration of sugar as IVIG. Thus the medical and the nursing staff did not know whether the baby received IVIG or 5% dextrose. The infusion materials were prepared by the pharmacist who also kept the randomisation codes, and were given to the doctor responsible for the study in a syringe so that the clinician supervising the infusion and those in charge of the patients remained blind to the products received. Thus double blind randomisation was maintained. The first infusion was given within the first two days of life.

The doses of IVIG (or placebo) were administered slowly using a syringe pump at an initial rate of 0.6 ml/Kg/hour (0.5 μg/Kg/minute of IVIG in IVIG group) for 30 minutes. Then the dose was increased to 3.6 ml/Kg/hour (3 μg/Kg/minute of IVIG) provided there had been no adverse reaction to the infusion, such as change in heart rate, respiratory rate or blood pressure. These measures were recorded every 30 minutes during the infusion period and one hour after.

Blood was taken from the infants before the infusion for the biochemical study (sodium, potassium, osmolality, sugar, urea, serum creatinine and serum bilirubin), full blood count, blood gases and acid base balance (these were done routinely in the babies in the intensive care unit). 0.8 ml of blood was also taken for IgG and opsonisation studies. Half way through the infusion, blood was also taken for blood gases and acid base balance. One hour after the infusion was completed, 0.8 ml of blood was taken for IgG, biochemical study and blood gases and acid base balance. Urine was tested for protein and blood before the infusion and the day after.

The next day, at 7 days and 14 days post-infusion, blood was taken for full blood count, and biochemical studies of hepatic and renal function, so that acute and late onset side effects of IVIG could be evaluated. Other blood samples (0.8 ml) were collected to check IgG levels and opsonic activity. The blood which was taken for IgG and opsonisation was centrifuged and the serum was taken. The serum for IgG was kept at -20 ^{0}C. Serum for opsonisation was heated in a water bath at 56 ^{0}C for 30 minutes to remove the effect of the complement, then kept at -20 ^{0}C till the tests were done. All sera from a single patient were tested for IgG on the same day. Samples from a single patient were tested for opsonisation on the same day also so that the same source of neutrophils was used.

The patients were followed during the period of hospital stay for evidence of infection such as deterioration of their general condition, temperature instability, lethargy, vomiting, diarrhoea, abdominal distension, apnoea, tachypnoea, episodes of desaturation, hypotension, irritability, seizures, raised C-reactive protein, low platelet counts, high or low white blood cell counts or chest x- rays showing any evidence of infection. All of these signs and laboratory investigations were evaluated as possible signs of sepsis even if the blood, cerebrospinal fluid or urine culture were negative. If the baby showed clinical deterioration as mentioned before and the blood, cerebrospinal fluid or urine culture were positive for any micro-organisms causing an infection, this was considered as being confirmatory evidence of infection.

In addition to the above determinations, we measured the number of days during which an antibiotic was administered to the infants after enrolment in the study, regardless of the culture results. We also determined the number of days during which an infant required assisted ventilation. The presence of intracranial haemorrhage was determined using ultrasound evaluations for infants born at less than 32 weeks gestation in the first day of life and when clinically indicated.

0.8 ml of blood was taken for full blood count and IgG at the age of one, two and three months. If the infant was discharged from the hospital before all the blood samples had been collected, arrangements were made for the baby to be reviewed as an outpatient when the blood samples were collected.

Serum immunoglobulin was measured by Immunonephelometry assay in the Department of Biochemistry, Glasgow Royal Infirmary.

The study period of three months was selected as more than 95% of the late-onset sepsis appeared to occur during the first two months and the lowest level of IgG was reached by the age of three to six months.

2.3 Material for Prophylaxis

Human immunoglobulin for intravenous use

No target level of IVIG is known to prevent late onset bacterial sepsis in preterm babies and no known effective dose exists. In this study a dose of 500 mg/kg for those with birth weight 1001-1500 grams and a dose of 700 mg/kg to babies weighing less than 1000 grams was selected as previous studies suggested that these doses decreased bacterial infection. A higher dose of IVIG might suppress immunity by decreasing bacterial clearance and survival in infected animals[122], decreasing adult monocyte[123] and neonatal neutrophil[122] phagocytosis of bacteria in vitro. There are reports of fatal infection in immuno-compromised adults after high doses of IVIG [124].

Immunoglobulin used in this study was prepared by the Scottish National Blood Transfusion Service and consisted of a lyophilised preparation of IgG which was manufactured from unselected human plasma. It was prepared by conventional cold-

ethanol fractionation followed by ultra-filtration and mild pepsin proteolysis at pH 4. A wide range of in vitro tests including opsonisation, neutralisation and antigen-specific complement binding have shown that antibody function is well preserved and that all IgG subclasses are present. The preparation has been shown to contain antibodies against a wide range of viruses, bacteria and toxins.

Composition:

Each vial contains:

3 grams IgG.

6 grams sucrose.

Not more than 300 μg porcine pepsin.

All constituents are present in a lyophilised powder form. After reconstitution, the solution has a protein concentration of approximately 45 grams/L and approximately 90 grams/L of sucrose. More than 97% of the protein content is IgG. Each donated plasma used to manufacture the immunoglobulin preparation is carefully screened for any evidence of Hepatitis B, Hepatitis C, Human Immuno-deficiency Virus, or syphilis infections. The solution must be administered within four hours after reconstitution.

2.4 Neutrophil Oxidation, Metabolism and Degranulation (Chemiluminescence [CL])

Allen in 1972[156] noted that after the addition of particles or mico-organisms such as bacteria, yeast or zymosan to polymorphonuclear neutrophils, there was a faint light emission. He subsequently showed that this phenomenon correlated well with other parameters of oxidative metabolism and stimulation of the hexose-monophosphate shunt. Light producing chemical reactions by a non-thermal process

have been reported to occur after phagocytosis of opsonised particles by human polymorphonuclear neutrophils, eosinophils and monocytes. Such CL appears to be related to the generation of intermediate products of oxygen reduction such as singlet oxygen, super oxide and hydroxyl radicals which have been implicated as microbicidal agents.

In this phenomena (CL) the light is produced by a chemical reaction, the molecule responsible for emitting the light, absorbs free energy released by the reaction and becomes excited. In this state some of the peripheral electrons of the molecule are raised to a higher energy level. When these electrons lose energy they return to a lower energy level and the energy lost during this transition is emitted as photons, i.e. light. When the electrons have lost all their absorbed energy the molecule is then back in its stable state. The colour and wave length of the emitted light depend on several factors, including the type of molecule excited, the pH of the liquid medium, the presence of metal ions, the type of catalyst and the amount of free energy released by the chemical reaction.

The amount of light in CL is increased by the addition of soluble protein, such as the amino acids tyrosine and tryptophan, to the reaction medium. These agents appear to produce their effects not by increasing the rate of phagocytosis but by providing a substrate for secondary light-producing reactions which occur outside of the phagocyte.

The intensity of light emitted depends on the reaction rate of the oxido-reduction step which in turn depends on the concentration of molecules taking part in the reaction. Light intensity is therefore directly proportional to the concentration of the reactants. In practice only key substances such as adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate {ADN(P)H} and hydrogen peroxide

(H_2O_2) are measured directly by light measurement. CL has been used as a technique to investigate the de-granulation of polymorphonuclear neutrophils and their cytotoxic ability and it has become widely used as a replacement or alternative method for many assays carried out, such as catorimetry, spectrophotometry and the counting of radioactive emissions.

Advantages of CL

1. Higher sensitivity.
2. Wide measurement range.
3. Smaller specimens.
4. Safe to use, simple, lower cost instrumentation.
5. Highly specific, stable, non toxic reagent used providing rapid measurement.

Oxidative metabolism of other phagocytic cells can also be evaluated by the chemiluminescence method. Monocytes emit light intensity in approximately one third of that of neutrophils, and eosinophils emitting light with intensity equal to that of neutrophils. Luminescence analysis is based primarily on three light producing systems:

1. Firefly bioluminescence for the measurement of ATP.
2. Bacterial bioluminescence for the measurement of NAD(P)H.
3. Luminol chemiluminescence for the measurement of H_2O_2.

Luminol chemiluminescence

Luminol (5 amino 2,3 dihydro 1,4 phthalazenedine) is a synthetic compound which emits light when oxidized by either peroxides or oxygen radicals. It interacts with these oxidising species to produce larger, more measurable amounts of light at a peak wave length of approximately 425 nm. The reaction proceeds much faster in the

presence of certain metal ions and metal containing complexes such as haematin, the active group in haemoglobin.

Chemiluminescence assay

The PICO-LITE Model 6500 Luminometer system was used in our Chemiluminescence study. The PICO-LITE is an analytical instrument used to measure light (protons) emitted by biological and chemical reactions (bioluminescence and chemiluminescence). The PICO-LITE consists of a Detector and an Analyser which includes a printer.

The temperature was set at 37 ^{o}C at least 30 minutes before the run, and the computer was programmed as per protocol up to a starting point. 100 ul of opsonised E. coli or GBS and 50 ul of 10^{-5} M luminol were added to each vial which were placed in the darkened chambers of the luminometer (Packed Picolite-Model number 5600) which was kept at a temperature of 37 ^{o}C. Then 100 ul of PMN were added to each vial. A ratio of 50:1 of bacteria to PMN was found to give the optimum CL response. The CL was measured and was quantitated by the peak high of the response.

a. Isolation of polymorphonuclear cells from peripheral blood

Polymorphonuclear (PMN) cells were prepared from peripheral blood (blood samples were taken from healthy adult volunteers) using a modification of the method described by Boyum (1968). 7 ml of heparinised blood is added to 3 ml of 5% solution of Dextran in saline. After 30 minutes the PMNs rich plasma is layered gently into an equal volume of polymorphonuclear separating fluid (13.8% sodium metrizoate, 8% Dextran). The PMN layer is transferred into a tube in which PMNs are washed in Gel Hanks solution containing 0.1% gelatine. This is centrifuged at

1400 rpm for 5 minutes. The PMN cells are resuspended in Gel-Hanks solution at a concentration of $1x10^7$ ml^{-1}.

b. Preparation of bacteria

The bacterial preparation is used for the measurement of the killing ability of PMNs on opsonised bacteria with serum from the patient. 10 ml of Mueller- Hinton was inoculated with type III group B streptococci or E. coli (k1). These organisms were isolated from sick new-born babies who had died from septicaemia. The broths were incubated overnight at 37 ^{0}C. The bacteria were washed 3 times in sterile saline. The concentration of organisms was adjusted to $5x10^8$ colony forming units (CFU)/ml, using a Cecil spectrophotometer. An optical density of 0.45 at 620 nm corresponded to $5x10^8$ CFU/ml.

5×10^8 CFU quantity of bacteria (E. coli and group B streptococci) were preopsonised separately by incubation in 1 ml (equal volume) of 10% of the patient's serum diluted in Gel-Hanks (buffer) solution at 37 ^{0}C for 15 minutes in an orbital shaker. Optimum opsonisation of GBS and E. coli was found to be in 10% of human serum. There was no significant difference in opsonisation between 10% and 20% human serum.

The serum was collected from the 0.4 ml of blood which was taken from the neonate before the infusion of IVIG or 5% dextrose, 24 hours, 7 days and 14 days after each infusion. The serum was heated at 56 ^{0}C for 30 minutes to remove the effect of complement. All samples from each baby were done on the same day, so that the PMNs used were from the same donor. Therefore any difference in the CL results was due to the effects of the IgG in the serum of the different samples. After incubation for 15 minutes the opsonised bacteria were centrifuged at 3000 rpm for 15

minutes and the supernatant was discarded. The organisms were resuspended in the original volume of Gel-Hanks.

Statistics

Comparability of the demographic data of the IVIG treated and control group were assessed using the student's *t* test. Infection rates among the two study groups were compared by the chi-square test. All data were expressed as mean ± standard deviation. A p value of ≤ 0.05 was considered significant.

Chapter 3

Results

As noted from Table 2.1 there was no significant difference in the sex, gestational age, mode of delivery, Apgar score, duration of ventilation and use of surfactant between the IVIG and control group. There was no significant difference in the duration of antibiotic therapy between the two groups. The infants receiving IVIG were hospitalised for a mean period of 59.8 ± 36.6 days which was not significantly different from 56.9 ± 32.5 days in the control group. Also there was no significant difference in the total number of days during which infants required intensive care support. The mean period of intensive life support was 30.3 ± 24.8 days for IVIG recipients and 31.2 ± 23.7 in the control group.

Two babies in the control group died early in the study period and were excluded from the trial. Two babies in the IVIG group also died early in the study period and were excluded from the trial. Another one died at 32 days of age. He was included in the study until 14 days after the last IVIG infusion.

Number of deaths in the control group (Table 3. 1)

Case 1: Delivered at 26 weeks, had grade III IVH from day one, severe idiopathic respiratory distress syndrome and persistent pulmonary hypertension. On the second day he developed pulmonary haemorrhage. Despite continuing maximum life support, the baby died on day two.

Case 2: The second infant delivered at 24 weeks, had severe RDS, IVH grade IV from the first day, and a coagulopathy. PDA was treated with indomethacin. He had progressive ventricular dilatation and ventilatory support was withdrawn on day 12 when he was declared brain dead.

Both patient blood cultures were negative.

Number of deaths in the IVIG group (Table 3. 2)

Case 1: Preterm baby delivered by caesarean section at 26 weeks, had severe hypotension since birth leading to pre-renal failure, severe RDS complicated by recurrent pneumothoraces which ultimately led to respiratory failure and death at the age of 9 days. Blood culture at 8th day was positive for Staphylococcus epidermidis.

Case 2: Premature baby delivered at 26 weeks, had severe RDS which led to recurrent bilateral pneumothoraces treated by the insertion of a chest drain. The baby was also hypotensive and was found to have significant coagulopathy. Despite maximum support, the baby died from severe hypoxaemia at the age of 4 days. Blood cultures were negative.

Case 3: Premature baby delivered at 25 weeks who, despite steady and marked improvement in respiratory disease, had grade IV IVH/PVH from day 2 which led to convulsions and brain death. Respiratory support was withdrawn at the age of 34 days when the baby died.

Table 3.1 Clinical details of dead infants in IVIG group

Case No.	Sex	Mode of delivery	Birth weight (grams)	Ges. Age (weeks)	Apgar score at 5 min	Severity of RDS.	Ventilation (days)	IVH/PVH	Age at death (days)	Cause of death	Other
1	M	LUSCS	980	26	10	Sev.	9	-	9	RDS+ Pn.	Pn.+ Hyp.+ PDA
2	F	LUSCS	630	26	10	Sev.	4	-	4	RDS.+ Pn.	Pn. + Hyp.+ P.H.+ Coagulopathy
3	M	LUSCS	730	26	5	Mod.	38	Grade IV	34	Brain death	PDA +IVH + Con.

Table 3.2 Clinical details of dead infants in the control group

Case No.	Sex	Mode of delivery	Birth weight (grams)	Ges. Age (weeks)	Apgar score at 5 min	Severity of RDS.	Ventilation (days)	IVH/PVH	Age at death (days)	Cause of death	Other
1	M	LUSCS	1250	26	6	Sev.	2 Days	-	2	RDS+ P.P.H.	Hyp.+ P.H.
2	F	S.V.D.	800	24	8	Mod.	12 Days	Grade IV	12	RDS. Brain death.	PDA + Coagulopathy

PDA = Patent ductus arteriosus.

Pn. = Pneumothorax.

Hyp. = Hypotension.

P.P.H. = Persistent pulmonary hypertension.

P.H. = Pulmonary haemorrhage.

Con.= Convulsion.

3.1 Incidence of late onset sepsis

Late onset sepsis was defined as sepsis occurring at or after the age of five (5) days. Proven sepsis was defined as clinical symptoms and signs consistent with sepsis in association with isolation of a significant organism from a blood culture specimen, cerebrospinal fluid or bladder stab urine. Clinical signs consistent with sepsis were respiratory distress in the form of apnoea, tachyponea, increased oxygen requirement or a severe form requiring mechanical ventilation, hypotension, acidosis, temperature instability, lethargy, vomiting, diarrhoea, poor feeding abdominal distension, ileus, seizures, petechiae or purpuric rash and in a laboratory form as neutropenia, an immature to total neutrophil ratio of greater than 0.2, leucocytosis, chest x ray shows evidence of infection, high c- reactive protein and low platelets count.

Suspected episodes of sepsis were defined as clinical symptoms and signs suggesting sepsis or any investigation including chest X-ray showing a signs of infection but no micro-organisms isolated from blood, cerebrospinal fluid, or urine on culture.

In this study the infusion of IVIG was given every 14 days, due to the fact that exogenous intact gammaglobulin is rapidly catabolised by the neonates, with a half life of about 11-14 days (4 to 7 days for specific antibody), compared with an average half life of 3 weeks in adults. Among neonates with birth weight less than 1000 grams, doses as high as 900 mg/Kg every 2 weeks did not sustain a target level of 700 mg/dl of serum IgG for 14 days in 60% of infusions. Even among infants weighing more than 1000 grams, it was difficult to sustain serum levels for two weeks with doses of 500 to 900 mg/Kg of IVIG[114]. High doses of IVIG might suppress immunity by decreasing bacterial clearance. These studies indicated that the serum level was not maintained above the target level after 14 days of IVIG infusion.

Therefore the infused IVIG did not have a major role in the protection of preterm babies against bacterial infection after 14 days. In our study we divided the period during which infection could occur into two:

1. Within 14 days post infusion.
2. After 14 days post infusion.

Therefore any difference in the incidence of infection after 14 days post infusion period was due to chance rather than to the effect of the infused immunoglobulin.

a. Incidence of proven sepsis within the fourteen days post infusion period

Five (5) out of seventeen babies (17) in the IVIG group (29.4%) developed episodes of proven sepsis within 14 days post infusion period (Table 3.3) in comparison to nine (9) out of seventeen (17) babies (52.9%) in the control group. ($\chi^2 = 1.96$, $0.05 > p > 0.01$).

These results indicate that there is no statistical significant difference in the incidence of proven sepsis within 14 days post infusion between the IVIG group and the control group (Figure 3.1). Although, there was an overall reduction in sepsis in the IVIG group in comparison to the control group, these results are not statistically significant. The apparent reduction in the incidence of sepsis in IVIG group was noted mainly in babies with birth weight of $\leq$ 1000 grams. (Table 3.4).

In the group of babies (Figure 3.2) with birth weight $\leq$1000 grams, four (4) out of seven babies (7) in the IVIG group (57.1%) developed episodes of proven sepsis in comparison to seven (7) out of eight (8) babies (87.5%) in the control group. ($\chi^2 = 1.75$, $0.25 > p > 0.10$).

Although the IVIG group shows a decrease in the incidence of proven sepsis in comparison to the control group, this is not statistically significant.

Analysis of the number of episodes in babies with birth weight $\leq$ 1000 grams shows that 7 episodes of proven sepsis occurred in 4 infected babies in the IVIG group in comparison to 13 episodes in 7 infected babies in the control group ($p > 0.05$). There was no significant difference between the IVIG group and the control group in relation to the number of episodes.

There were no episodes of proven sepsis in babies with birth weight of 1001-1250 grams in either group. In the group of babies with birth weight 1251-1500 grams, one (1) baby out of four (4) in the IVIG group had episodes of proven sepsis as compared to two (2) out five (5) babies in the control group.
($\chi^2 = 0.223$, $0.75 > p > 0.50$).

There were two episodes of proven sepsis in one infected baby in the IVIG group in comparison to two episodes in two infected babies (one episode each) in the control group [$p > 0.05$].

These results indicate that there is no statistical significant difference in the incidence of proven sepsis or in the number of the episodes in this age group between IVIG and control groups.

In the whole group of babies with birth weight $\leq$ 1500 grams, five (5) out of seventeen (17) babies (29.4%) in IVIG group had a total of nine (9) episodes of proven sepsis within 14 days post infusion period in comparison to nine (9) out of seventeen (17) babies (52.9%) in the control group who developed 15 episodes [$p > 0.05$]. These results indicate that there is no statistically significant difference in

the number of episodes of proven sepsis between the IVIG group and the control in spite of an apparent overall difference. The episodes of proven sepsis occurred mainly after the age of five days in the control group.

Analysis of the causative micro-organisms (Table 3.5) in the IVIG group shows that babies with birth weight ≤1000 grams had seven (7) episodes of sepsis and all of them were due to Staphylococcus epidermidis, while only one baby with birth weight of 1251-1500 grams had two episodes of proven sepsis, one due to Candida septicaemia and the second one due to Enterobacter.

In the control group, 12 episodes out of 13 (92.3%) in babies with birth weight ≤ 1000 grams were due to Staphylococcus epidermidis and one out of 13 (7.6%) was due to Candida albicans. The two episodes in babies with birth weight of 1251-1500 grams were also caused by Staphylococcus epidermidis.

b. Incidence of proven sepsis after 14 days post infusion period

In the IVIG group five (5) out of sixteen (16) babies (31.2%) developed episodes of proven sepsis in comparison to three (3) out of seventeen (17) babies (17.6%)) in the control group (Table 3.3).
($\chi^2 = 0.82$, $0.50 > p > 0.25$).

These results indicate that there was no statistically significant difference in the incidence of proven sepsis after 14 days between the IVIG and control groups (Figure 3.2).

Table 3.3 Incidence of proven sepsis in IVIG and control groups.

Time of proven sepsis	IVIG group	Control group
Within 14 days	5/17 (29.4%)	9/17 (52.9%)
After 14 days	5/16 (31.2%)	3/17 (17.6%)

Table 3.4 Incidence of proven sepsis within 14 days post-infusion at different birth weights in IVIG and control groups.

Birth weight	IVIG group	Control group
≤1000 grams	4/7 (57.1%)	7/8 (87.5%)
1000 - 1250 grams	0/6	0/4
1251 - 1500 grams	1/4 (25%)	2/5 (40%)

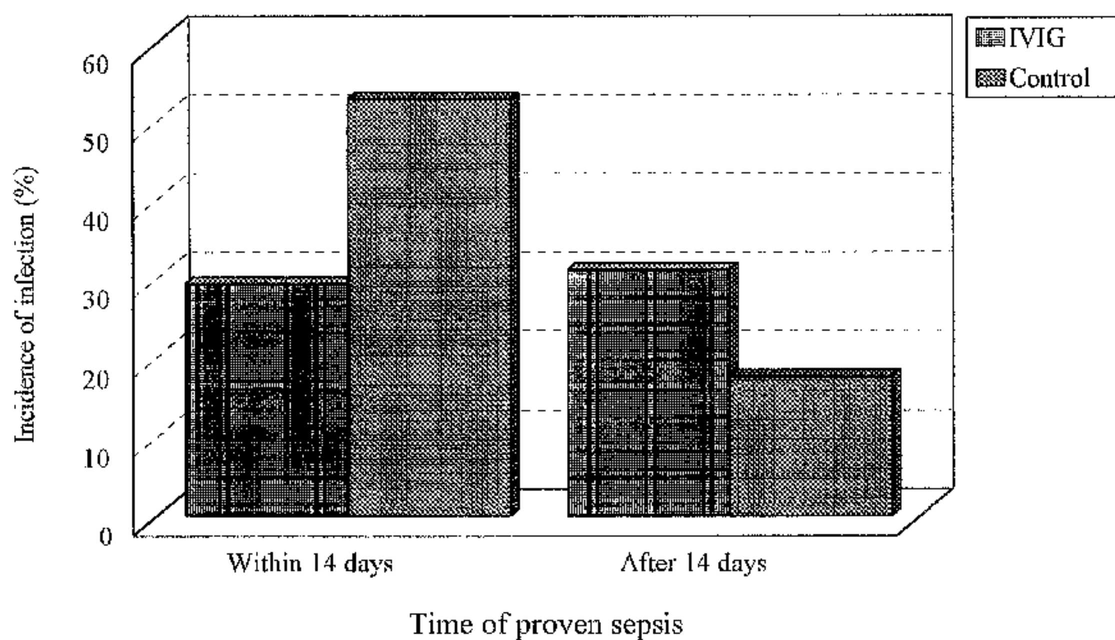

Figure 3.1 Incidence of proven sepsis in the IVIG and control groups

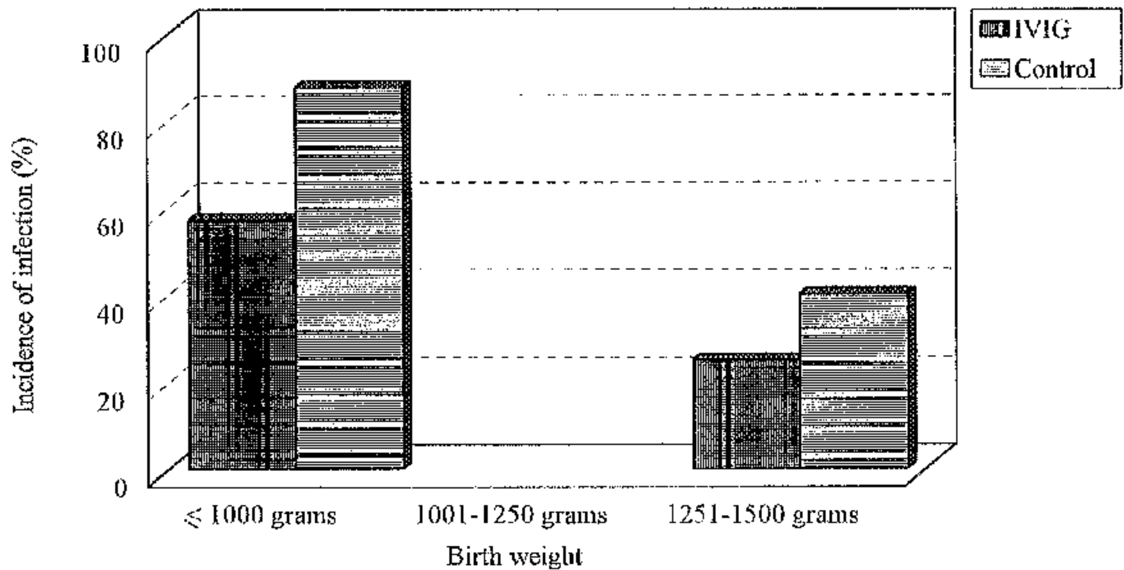

Figure 3.2 Incidence of proven sepsis within 14 days in the IVIG group at different birth weights

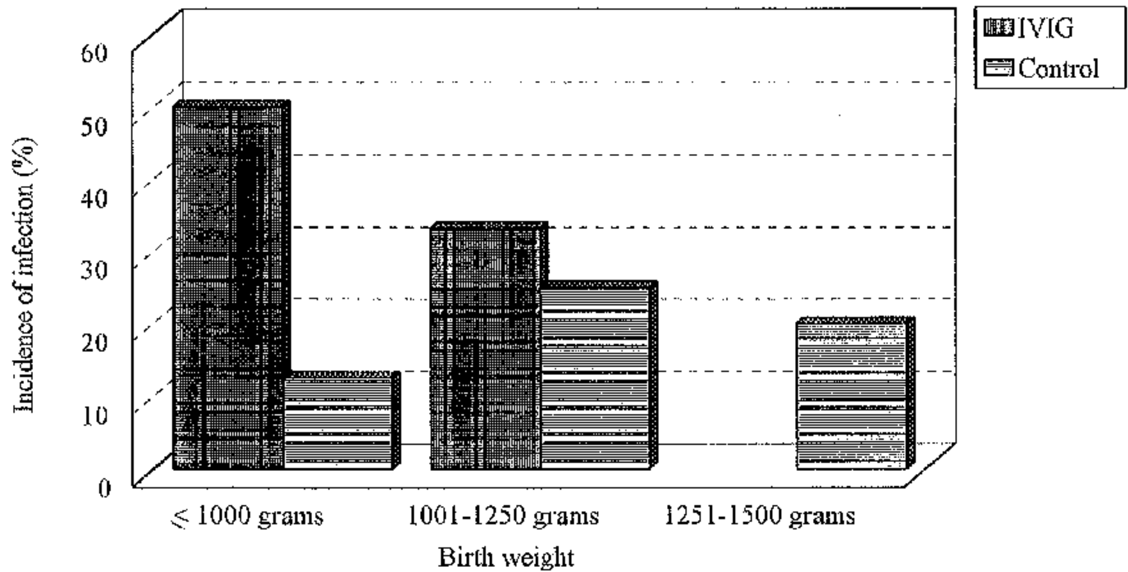

Figure 3.3 Incidence of proven sepsis after 14 days post infusion in IVIG and control groups at different birth weight

The analysis of the incidence of proven sepsis after 14 days post infusion in different birth weight groups indicates that there is no significant difference between the two groups. One (1) out of eight (8) babies (12.5%) in the control group with birth weight $\leq$ 1000 grams in comparison to three out of six (50%) in IVIG group had episodes of proven sepsis (Table 3.6)
($\chi^2 = 2.36,\ 0.25 > p > 0.10$).

One (1) out of four (4) babies (25%) in the control group with birth weight 1001-1250 grams had an episode of sepsis in comparison to two (2) out of six (6) babies (33.3%) in the IVIG group.
($\chi^2 = 0.075,\ 0.90 > p > 0.75$).

In the group of babies with birth weight of 1251-1500 grams, no babies in the IVIG group had episodes of proven sepsis while one (1) out of five (5) babies in the control group (20%) had an episode of proven sepsis after 14 days post infusion.
($\chi^2 = 0.897,\ 0.50 > p > 0.25$).

These results indicate that there is no significant difference in proven sepsis between babies of different birth weight in the IVIG and control groups.

Five (5) episodes of proven sepsis occurred in the five infected babies in the IVIG group in comparison to six (6) episodes in three infected babies in the control group ($p > 0.05$). That means there is no significant difference in the number of the episodes between the two groups.

All of the episodes in the control group were due to Staphylococcus epidermidis and occurred at the age of 17 to 50 days.

In the IVIG group four 4 out of 6 episodes were due to Staphylococcus epidermidis and one was due to Enterococcus (streptococcus) faecalis.

In the IVIG group the episodes of proven sepsis occurred between the age of twenty (20) days and seventy six (76) days. Three babies had four (4) episodes of Staphylococcus epidermidis septicaemia. One baby had Enterococcus faecalis septicaemia at age of 74 days. Table 3.5 shows the causative micro-organisms in both groups up to three months of age.

Twenty one episodes of proven sepsis occurred in babies in the control group within this three month period, twenty of which were due to S. epidermidis and one due to C. albicans. Fourteen out of these 21 episodes (66.6%) were associated with the presence of a long line. Fourteen episodes of proven sepsis occurred in the IVIG group, eleven out of these fourteen were due to S. epidermidis and six (42.8%) associated with the presence of a long line. Therefore 57.1% of infection episodes were associated with the presence of a long line.

Twelve out of 35 episodes (34.2%) of proven sepsis were accompanied by raised C- reactive protein. Two episodes were due to Candida albicans and 10 episodes were due to S. epidermidis. In the remaining 23 episodes C- reactive protein was not raised. C- reactive protein was raised to a high level (it reached 125 mg/dl in one case) in infection due to C. albicans, while in infection with S. epidermidis the C-reactive protein usually raised to a lower level but only in two cases it was raised to 81 and to 86 mg/l.

It is noted that most of the infants in this study did not develop infections with organisms widely recognised as pathogens in neonates, such as GBS and enteric gram negative organisms. Rather, the infecting micro-flora represented organisms

Table 3.5 Causative micro-organisms in IVIG and control groups within a 3 month period.

Micro-organisms	IVIG group	Control group	Total (IVIG + control)
S. Epidermidis	78.6%	95.2%	88.5%
Candida albicans	7.1%	4.8%	5.7%
Enterobacter	7.1%	—	2.9%
Enterococcus faecalis	7.1%	—	2.9%

Table 3.6 Incidence of proven sepsis after 14 days post-infusion at different birth weights in IVIG and control groups.

Birth weight	IVIG group	Control group
≤1000 grams	3/6 (50%)	1/8 (12.5%)
1001 -1250 grams	2/6 (33.3%)	1/4 (25%)
1251 - 1500 grams	0/4	1/5 (20%)

commonly encountered in nosocomial infection in other inpatient intensive care population. The commonest was S. epidermidis.

c. Incidence of suspected sepsis within fourteen (14) day post infusion period

The definition of suspected sepsis means that the baby was unwell, had signs suggestive of sepsis and antibiotics were given but cultures of blood, cerebrospinal fluids or urine were negative.

In the IVIG group ten (10) out of seventeen (17) babies (58.8%) developed episodes of suspected sepsis within the 14 day post-infusion period (Figure 3.4) in comparison to eleven (11) out of seventeen (17) babies (64.7%) in the control group (Table 3.7).
($\chi^2 = 0.122$, $0.75 > p > 0.50$).

These results indicate that there is no significant difference in the incidence of suspected sepsis within 14 days post-infusion between the IVIG and control group. But there is a significant difference in the number of the episodes of suspected sepsis between the two groups ($p < 0.05$). In the control group 26 episodes of suspected sepsis occurred in comparison to 13 episodes in IVIG group.

In the IVIG group (Table 3.8) six (6) out of seven (7) babies (85.7%) with birth weight $\leq$1000 grams in comparison to eight (8) out of eight (8) babies (100%) in the control group had episodes of suspected sepsis within 14 days post infusion (Figure 3.4), ($\chi^2 = 1.209$, $0.25 > p > 0.10$).

Babies in the control group had 23 episodes in comparison to 9 episodes in IVIG group ($p < 0.05$) , which is statistically significant.

Two out of six babies (33.3%) with birth weight 1001-1250 grams in the IVIG group had episodes of suspected sepsis (two episodes) in comparison to none in the control group. That means there is no significant difference between the two groups. ($\chi^2 = 1.666$, $0.25 > p > 0.10$).

In the control group three out of five babies (60%) with birth weight 1251-1500 grams in comparison to two out of four babies (50%) in IVIG group had an episode of suspected sepsis. There is no significant difference between the two groups. ($\chi^2 = 0.086$, $0.90 > p > 0.75$).

In this age group, babies in the IVIG group had 3 episodes in comparison to 4 episodes of suspected sepsis in control group ($p > 0.05$). That means no significant difference between the two groups.

d. Incidence of suspected sepsis after the 14 day post infusion period

Seven out of 16 babies (43.7%) in the IVIG group had episodes of suspected sepsis (Table 3.7) in comparison to two out of 17 babies (11.7%) in the control group after the 14 days post infusion period (Figure 3.4), ($\chi^2 = 4.25$, $p < 0.05$).

These results indicate that there is a statistically significant difference between the two groups against the IVIG group as more babies in this group had episodes of suspected sepsis. Table 3.9 shows the incidence of suspected sepsis after the 14 days post-infusion period at different birth weights in both groups (Figure 3.6).

Table 3.7 Incidence of suspected sepsis in the IVIG and control group.

Time of suspected sepsis	IVIG group	Control group
Within 14 days	10/17 (58.8%)	11/17 (64.7%)
After 14 days	7/16 (43.7%)	2/17 (11.7%)

Table 3.8 Incidence of suspected sepsis within 14 days post-infusion in the IVIG and control groups matched by birth weight group.

Birth weight	IVIG group	Control group
≤1000 grams	6/7 (85.7%)	8/8 (100%)
1001 -1250 grams	2/6 (33.3%)	0/4
1251 -1500 grams	2/4 (50%)	3/5 (60%)

Table 3.9 Incidence of suspected sepsis after 14 days post infusion at different birth weights in the IVIG and control groups.

Birth weight	IVIG group	Control group
≤ 1000 grams	2/6 (33.3%)	0/8
1001 -1250 grams	4/6 (66.6%)	0/4
1251 -1500 grams	1/4 (25%)	2/5 (40%)

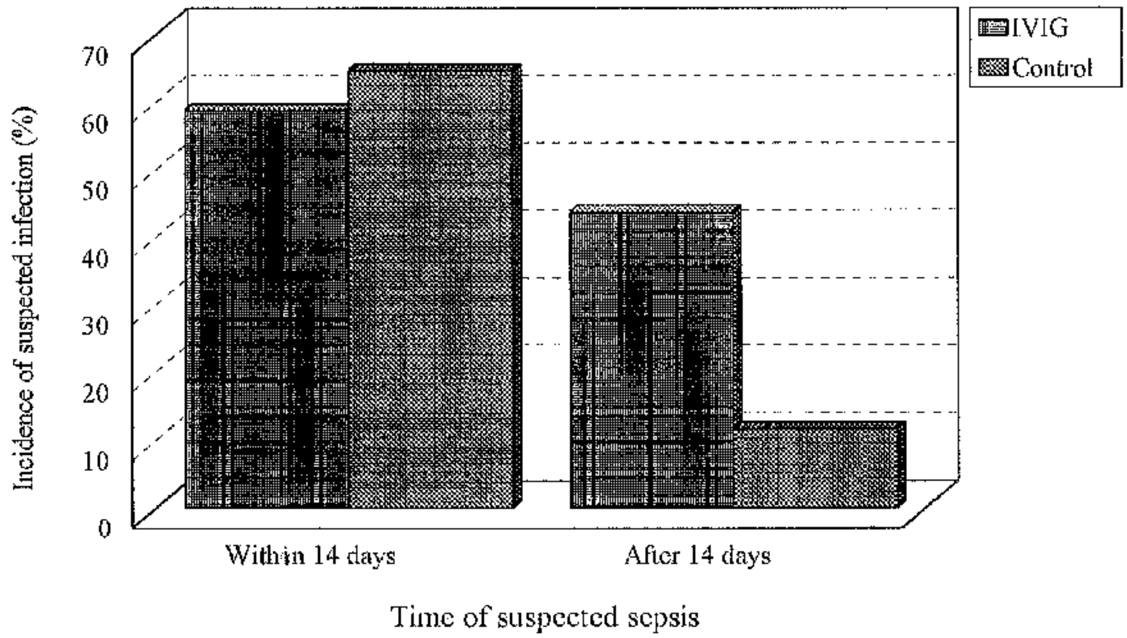

Figure 3.4 Incidence of suspected sepsis in the IVIG and control groups

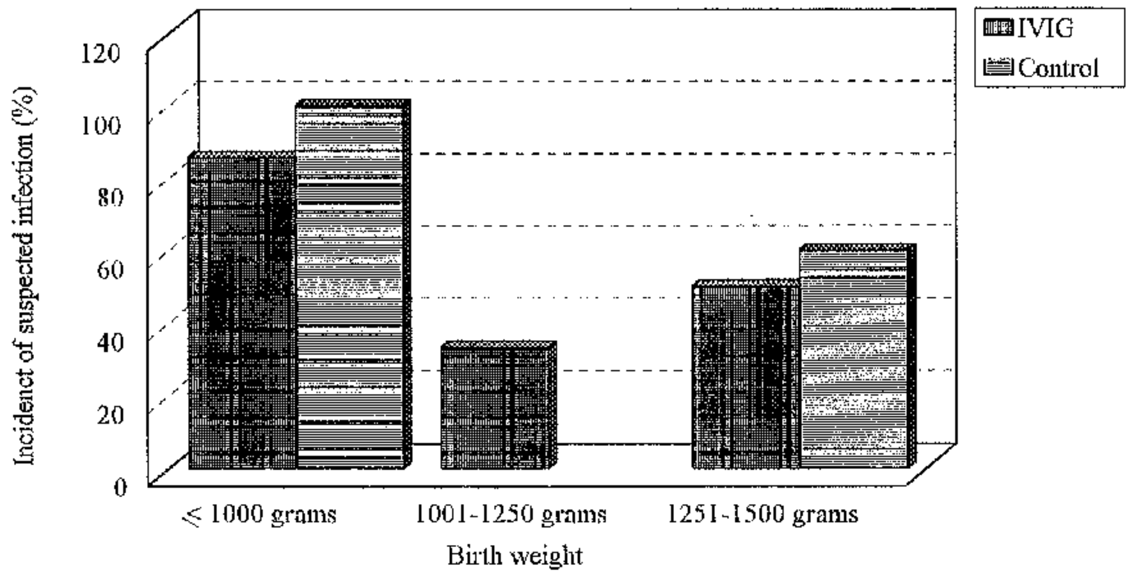

Figure 3.5 Incidence of suspected sepsis within 14 days post infusion in the IVIG and control groups at different birth weights

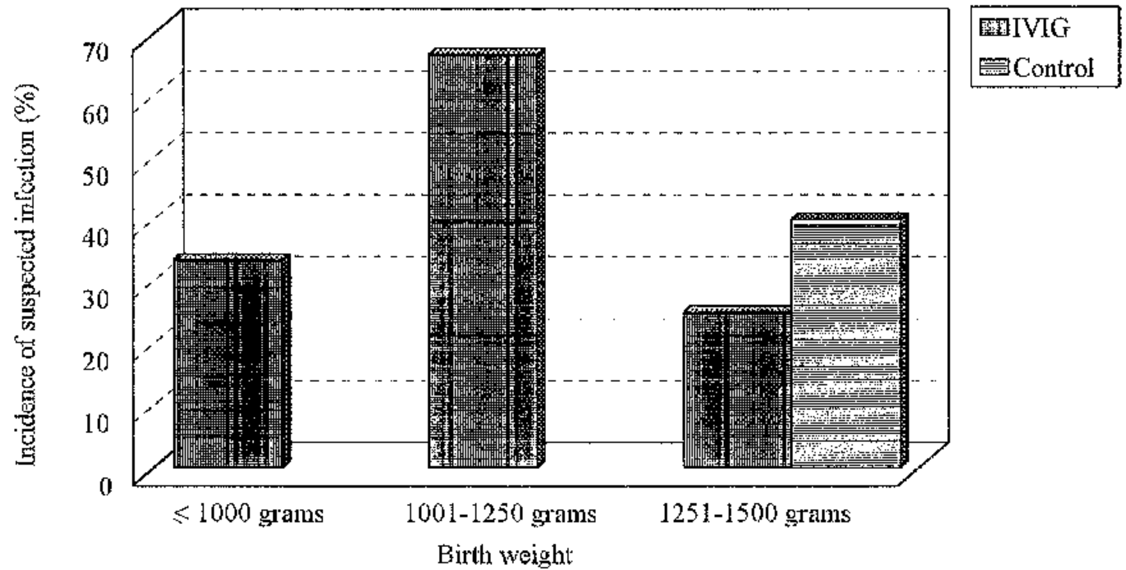

Figure 3.6 Incidence of suspected sepsis after 14 days post infusion in the IVIG group at different birth weight

There is no statistically significant difference in the number of the episodes between the two groups. Seven babies had eleven episodes in the IVIG group in comparison to three episodes in two babies in the control group ($p > 0.05$).

There was no significant difference (Table 3.10) in the number of babies fed breast milk, formula milk or a mixture of the two between the IVIG and control groups. Also there was no significant difference in the incidence of proven septic episodes and suspected necrotising enterocolitis between babies fed breast milk, formula milk or a mixture of the two in both study groups (Table 3.11).

Three babies in the IVIG group developed signs of suspected necrotising enterocolitis. One was fed breast milk, one formula milk, and the third fed a mixture of the two. Two babies in the control group developed signs of suspected necrotising enterocolitis, one was fed breast milk and the second fed formula milk.

As noted from these results, breast milk in our study did not seem to make any difference to the incidence of infection episodes or suspected necrotizing enterocolitis.

Table 3.10 Type of feeding in the IVIG and control groups

Type of feeding	IVIG group	Control group
Breast feeding	3/17	1/17
Formula feeding	6/17	8/17
Mixed feeding	8/17	8/17

Table 3.11 Incidence of infection in relation to the type of feeding

Feeding/infection	IVIG group	Control group
Breast feeding	1/3	1/1
Formula feeding	2/6	4/8
Mixed feeding	2/8	4/8

3.2 Effect of intravenous immunoglobulin on serum IgG level

At the study entry the mean serum concentration of IgG in the IVIG group was 456 ± 162 mg/dl (range: 240 - 770 mg/dl) and 467 ± 37 mg/dl (range: 310 - 740 mg/dl) in the control group. These results indicate that there was no significant difference in the mean serum IgG concentration at the entry of the study, $p > 0.05$ (Figure 3.12). After the first infusion the mean concentration of IgG was significantly increased in the IVIG group throughout the first 14 days ($p < 0.001$). There was no significant change in the control group IgG concentration one hour after the initial infusion whereas the mean serum IgG concentration in IVIG group increased by 230% from the pre-infusion level.

Although the mean serum IgG concentration had decreased to 72 % of the peak post-infusion level by 24 hours in IVIG group, it still remained 166 % above the baseline. At 7 days the mean serum IgG concentration in the IVIG group was 149.3% above baseline (decreased to 65 % of the peak post-infusion level) and at 14 days it was 121.7 % above baseline (decreased to 53% of the peak post infusion in the IVIG group).

By the end of the second week, five (5) babies in IVIG group still needed intensive life support as did another 5 babies in the control group. These babies received a second infusion.

The mean serum IgG pre-infusion level (Table 3.13) was 574 ± 216 mg/dl (range: 320-880 mg/dl) in the IVIG group in comparison to 422 ± 89 mg/dl (range: 350-550 mg/dl) in the control group. These results indicate that there was no significant difference in the mean IgG concentration between the two groups, $p > 0.05$ (Figure 3.8).

Throughout the first 14 days after the second infusion, the mean serum IgG concentration in the IVIG group was significantly increased in comparison to the changes in the mean serum IgG concentration in the control group ($p < 0.001$).

There was no significant change in the mean serum IgG concentration in the control group one hour after the infusion whereas the mean serum IgG concentration in the IVIG group increased by 210 % from the pre-infusion level.

By 24 hours post-infusion, the mean serum IgG concentration in the IVIG group had decreased to 81% of the peak post-infusion level, but this is still above the baseline by 171 %. At the 7th day the mean serum IgG concentration in the IVIG group was 116.7 % above the baseline (55% of the peak post-infusion level) and by 14 days it was 102.7 % above the baseline level (49% of the peak post-infusion level).

By the age of one month two babies in each group were still receiving intensive life support, and a third dose was given to them.

After the third infusion (Table 3.14) the mean serum IgG concentration was significantly increased in the IVIG group throughout the 14 days period post-infusion, $p < 0.001$ (Figure 3.9).

There was no significant change in the control group IgG concentration one hour after the infusion, whereas the mean serum IgG concentration in the IVIG group increased by 220% from the pre-infusion level.

Although the mean serum IgG concentration had decreased to 79 % of the peak post-infusion level by 24 hours in the IVIG group, it still remained 175.8 % above the baseline.

At day 7 the mean serum IgG in the IVIG group was 141.3 % above the baseline (63% of the peak post infusion level) and at 14 days it was 125.5 % above the baseline (56% of the peak post-infusion level).

One baby in the IVIG group, a second twin delivered at 28 weeks of gestation, was ventilated for 55 days. She needed a 4th infusion of IVIG at the age of 46 days. No baby in the control group needed a fourth infusion.

The pre-infusion IgG concentration (Table 3.15) was 580 mg/dl. This increased by 190% one hour after the infusion. After 24 hours the mean serum IgG concentration decreased to 76% of the peak post infusion level, but it still remained 144.8 % above the baseline. By 7 days the mean serum IgG concentration decreased further to 74% of the peak post infusion level, although it still remained 141.4 % above the baseline. At 14 days the mean was 129.3 % above the baseline (68% of the peak post-infusion level) {Figure 3.10}.

There was a significant difference between the two groups (Table 3.16) in the mean concentration of IgG during the first and the second months of life, but there was no significant difference at the age of three months.

At the age of one month the mean serum IgG concentration in the IVIG group was 413 ± 167 mg/dl (range: 280-670 mg/dl) in comparison to 273 ± 63 mg/dl (range: 170-370 mg/dl) in the control group ($p < 0.05$). These results indicate a statistically significant difference between the IVIG group and control group in the mean IgG concentration at the age of one month.

At the end of the second month of life, the mean IgG concentration in the IVIG group was 331 ± 183 mg/dl (range: 160-760) in comparison to 152 ± 49 mg/dl (range: 100-230) in the control group ($p < 0.05$).

That means there is a significant difference in the mean IgG concentration between the control and IVIG groups.

The mean concentration of IgG in the IVIG group was slightly higher than that in the control group at the age of three months. It was 218 ± 90 mg/dl (range: 100-670 mg/dl) in comparison to 113 ± 32 mg/dl (range: 60-160 mg/dl) in the control group ($p > 0.05$). This was not statistically significant, (Table 3.16).

Table 3.17 and Table 3.18 shows the mean concentration of IgG in the control group and IVIG group at the age of 1, 2 and 3 months in babies of different birth weights.

The mean IgG concentration at the age of one month (Table 3.19) in those babies who received only one dose of IVIG was 338 ± 102 mg/dl (range: 100 - 470 mg/dl) in comparison to 262 ± 63 mg/dl (range: 170 - 340 mg/dl) in the control group (in the group of babies who were chosen from the whole group with matched gestational age), $p> 0.05$.

At the age of two months the mean serum IgG concentration in the IVIG group was 241 ± 71 mg/dl (range: 160-380 mg/dl) in comparison to 153 ± 57 mg/dl (range: 100-230 mg/dl) in the control group ($p < 0.05$).

Table 3.12 Effect of IVIG on serum concentration of IgG (before and after the first infusion).

Time	IgG Concentration (mg/dl)	
	Control group	IVIG group
Before infusion	467 ± 137	456 ± 162
1 hour after infusion	492 ± 141	1045 ± 253 *
24 hours after infusion	508 ± 146	757 ± 201 *
7 days after infusion	484 ± 119	681 ± 140 *
14 days after infusion	402 ± 120	555 ± 148 *

n = 17 (control group).

n = 17 (IVIG group).

* = significant.

Table 3.13 Effect of IVIG on serum concentration of IgG (before and after the second infusion).

Time	IgG Concentration (mg/dl)	
	Control group	IVIG group
Before infusion	422 ± 89	574 ± 216
1 hour after infusion	412 ± 71	1208 ± 276 *
24 hours after infusion	404 ± 89	982 ± 221*
7 days after infusion	368 ± 79	670 ± 122*
14 days after infusion	284 ± 57	590 ± 146*

n = 5 (control group).

n = 5 (IVIG group).

* = significant.

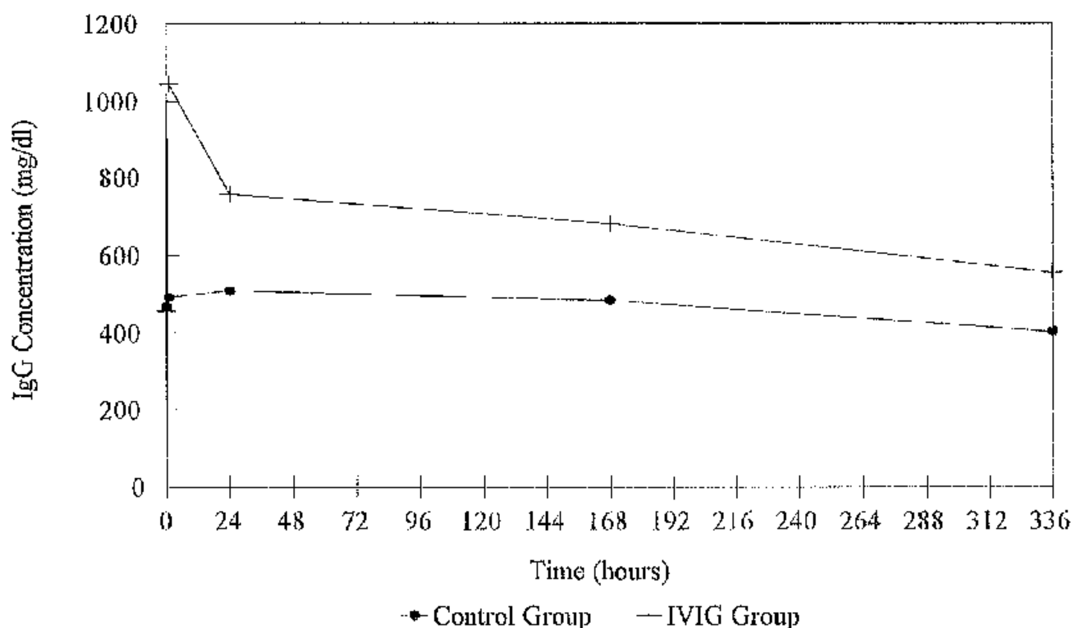

Figure 3.7 Serum IgG concentration in the IVIG and control group before and after the first infusion

n_1 (control) =17

n_2 (IVIG) = 17

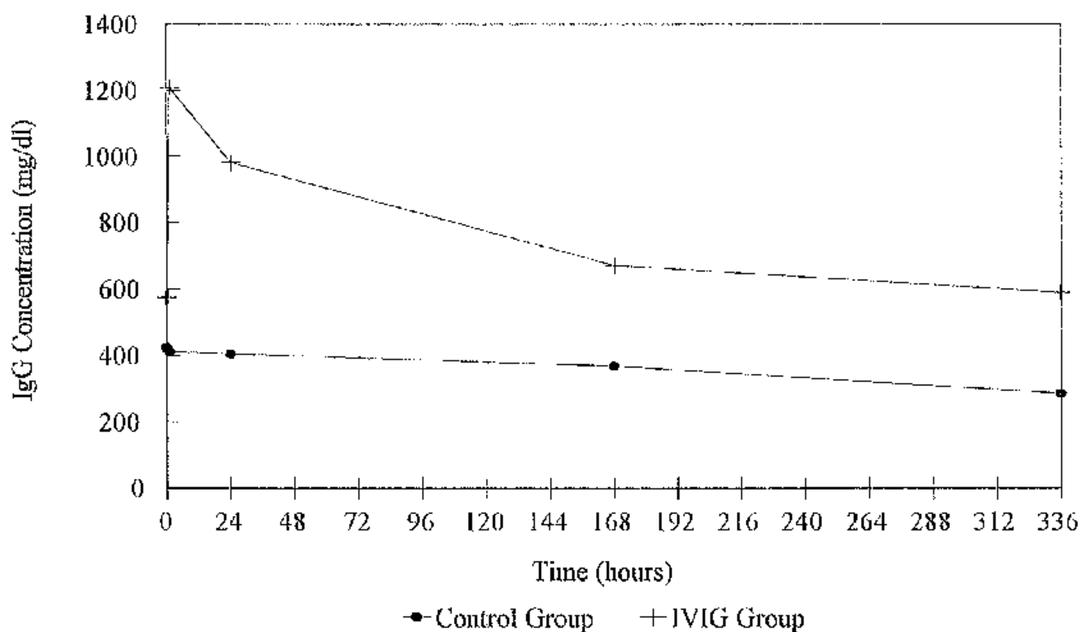

Figure 3.8 Serum IgG concentration in the IVIG and control group before and after the second infusion

n_1 (control) = 5

n_2 (IVIG) = 5

Table 3.14 Effect of IVIG on serum concentration of IgG (before and after the third infusion).

Time	IgG Concentration (mg/dl)	
	Control group	IVIG group
Before infusion	285 ± 07	506 ± 120*
1 hour after infusion	300 ± 00	1120 ± 173*
24 hours after infusion	380 ± 56	890 ± 160*
7 days after infusion	295 ± 07	715 ± 205*
14 days after infusion	280 ± 28	635 ± 77*

n = 2 (control group).

n = 2 (IVIG group).

* = significant.

Table 3.15 Effect of IVIG on serum concentration of IgG before and after the fourth infusion (no one in the 5% dextrose group received the fourth dose).

Time	Serum IgG concentration (mg/dl)
Before	580
After 1 hour	1100
After 24 hours	840
After 7 days	820
After 14 days	750

n = 1

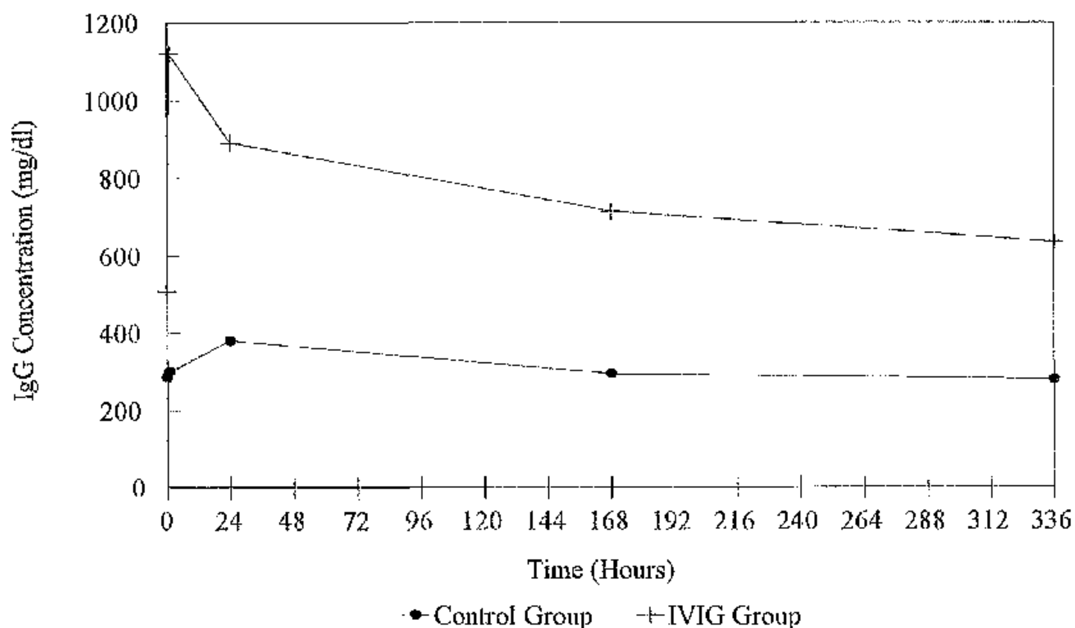

Figure 3.9 Serum IgG concentration in the IVIG and control group before and after the third infusion

n_1 (control) = 2

n_2 (IVIG) = 2

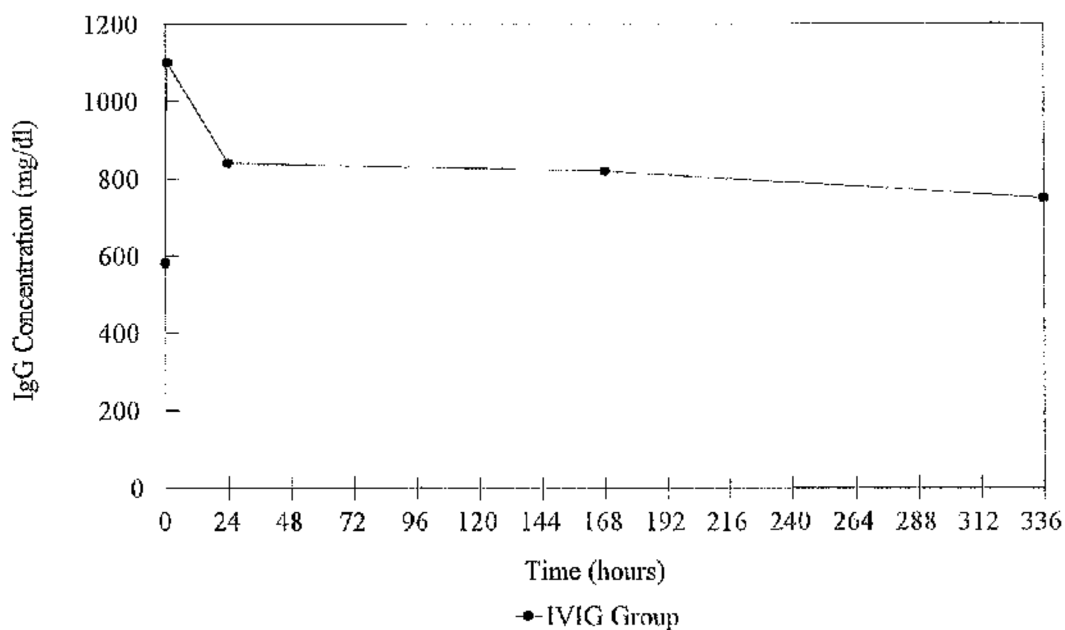

Figure 3.10 Serum IgG concentration in the IVIG group before and after the fourth infusion

n(IVIG)=1

At 3 months of age the mean IgG concentration in the IVIG group was 243 ± 72 mg/dl (range: 160-260 mg/dl) in comparison to 122 ± 31 mg/dl (range: 93 - 160 mg/dl) in the control group ($p < 0.05$).

These results indicate that there is no significant difference in the mean IgG concentration at one month between control and IVIG groups, although the mean IgG concentration in the IVIG group is higher than that in the control group. But at the age of two and three months there was a significant difference in the mean IgG concentration between the IVIG and control group (Table: 3. 16).

Maternal IgG crosses the placenta predominantly in the later part of the third trimester of pregnancy, so that, at birth, serum IgG levels in premature infants are well below levels observed in the term infants. Levels of serum IgG at birth are directly proportional to gestational age and their decline during the first months of life results in the preterm infants becoming severely hypogammaglobulinaemic. Table 3.20 show the serum IgG concentration at different gestational ages in the control group babies (Figure 3.11) and Table 3.21 shows serum IgG concentration at different post-natal ages.

3.3 Serum opsonic activity of E. coli and GBS

In this study the opsonic activity of the premature infant's serum for E.coli and GBS were studied in both the IVIG and control group. Neutrophils from adult volunteers were used to eliminate the effect of poor neutrophil function in preterm infants. Due to the difference in the sources of neutrophil cells used (different volunteers) throughout the study period it is difficult to compare the high peak value of serum opsonic activity between the babies in the two groups. Therefore the serum

Table 3.16 Mean serum concentration of IgG (mg/dl) at 1, 2 and 3 months in the IVIG and control groups.

Time	Treatment group	Control group
1 month	413 ± 167 (range: 280-670)	273 ±63 (range: 170-370)
2 months	331 ± 183 (range: 160-750)	152±49 (range: 100-230)
3 months	218 ± 90 (range: 100-670)	113 ±32 (range: 60-160)

Table 3.17 Mean serum IgG concentration (mg/dl) at 1, 2 and 3 months at different birth weights in the control group.

Time/Birth weight	≤ 1000 grams	1001—1250 grams	1251—1500 grams
1 month	264 ± 63 (range: 170-340)	320 ± 197 (range: 100-230)	260 ± 88 (range: 140-340)
2 months	154 ± 61 (range: 100-230)	180 ± 56 (range: 140-220)	160 ± 101 (range: 120-300)
3 months	116 ± 26 (range: 100-160)	170 ± 70 (range: 120-300)	144 ± 84 (range: 160-280)

Table 3.18 Mean serum IgG concentration (mg/dl) at 1, 2 and 3 months at different birth weights in the IVIG group.

Time/Birthweight	≤ 1000 grams	1001—1250 grams	1251—1500 grams
1 month	492 ± 180 (range: 300-670)	293 ± 117 (range: 160-470)	466 ± 85 (range: 360-470)
2 months	380 ± 240 (range: 160-750)	237 ± 43 (range: 200-300)	376 ± 175 (range: 200-550)
3 months	206 ± 121 (range: 100-360)	240 ± 45 (range: 200-290)	210 ± 20 (range: 205-216)

Table 3.19 Effect of one dose of IVIG on serum IgG concentration in the IVIG group in comparison to serum IgG concentration in control group (babies with matched gestational age).

Age	IgG concentration mg/dl	
	IVIG group	Control group
1 month	338.18 ± 102.45 (range:100-470)	262.5 ± 63.18 (range: 170-340)
2 months	241.11 ± 71.14 (range:160-380)	153.33 ± 57.85 (range:100-230)
3 Months	242.66 ± 71.46 (range: 160-260)	122.33 ± 30.91 (range: 93-169)

Table 3.20 Serum IgG concentration at different gestational ages.

Gestation Age	IgG Concentration (mg/dl)
24 - 25 weeks	320 ± 79
25 - 26 weeks	420 ± 0
26 - 27 weeks	350 ± 71
27 - 28 weeks	460 ± 110
28 - 29 weeks	541 ± 132
29 - -30 weeks	465 ± 176
30 - 31 weeks	580 ± 164
31 - 32 weeks	442 ± 76

Table 3.21 Mean serum IgG concentration in the control group at different birth weights and post natal ages.

Birth weight	Age in days					
in grams	2	9	16	30	60	90
≤ 1000	460 ± 146	450 ± 130	383 ± 138	264 ± 63	154 ± 61	116 ± 26
1001-1250	527 ± 175	500 ± 98	400 ± 148	326 ± 193	180 ± 56	170 ± 70
1251-1500	432 ± 96	528 ± 119	434 ± 78	260 ± 88	183 ± 101	144 ± 84

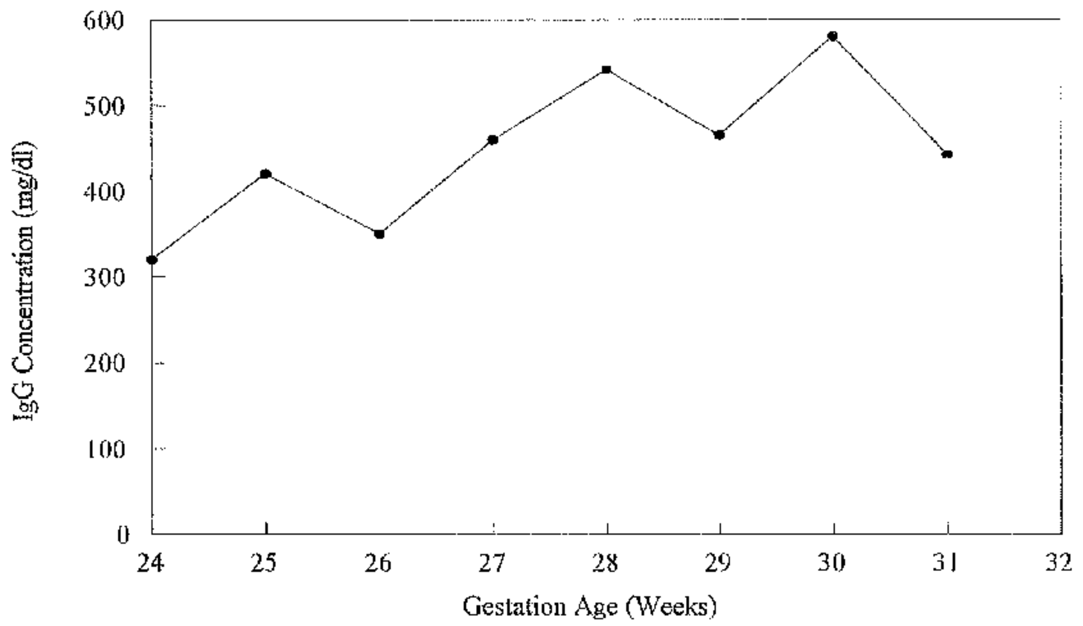

Figure 3.11 Serum IgG concentration at different gestational ages (control group)

opsonic capacity at entry to the study was considered as 100% and the serum opsonic capacity at different times after the infusion of either IVIG or 5% dextrose were calculated as a percentage rather than a value and compared to the 100% of the pre-infusion level.

There was no significant difference (Tables 22-25) in the mean value of GBS opsonisation between the IVIG and control groups during the study period, $p > 0.05$ (Figure 3.12).

There was no significant difference (Tables 26 - 29) in the E. coli opsonisation between the IVIG group and control group during the study period time ($p > 0.05$). Although the mean serum opsonic capacity for E. coli in the IVIG group especially during the second and third infusion study period was higher than that in the control group, the difference did not reach a significant level (Figure 3.13).

There was no significant difference (Tables 30 - 37).in the mean serum opsonic capacity for E. coli and GBS between the infected and non-infected babies in either IVIG group or the control group at any time during the study period ($p > 0.05$). This is shown in Figures 3.14 - 21.

The comparison of the serum opsonic capacity of E.coli and GBS in the IVIG and control groups measured during the pre-infusion and post-infusion period at different times during the study period (at 1,7,14 days) reveals no significant difference ($p > 0.05$).

From the results of our study, there was no correlation between the serum opsonic capacity of E.coli and GBS, IgG concentration at different times during the study period and the time of infection episodes with certain micro-organisms.

Table 3.22 Serum opsonisation of GBS (mean ± SD) in IVIG and control groups during the first infusion period

Time	IVIG group	Control group
Before	100%	100%
24 hours	95.7 ± 37%	106.2 ± 30%
7days	108.2 ± 51%	109.58 ± 32%
14 days	115.8 ± 104%	118.72 ± 63%

n_1 (IVIG) =17

n_2 (control) = 17

Table 3.23 Serum opsonisation of GBS (mean ± SD) in IVIG and control groups during the second infusion period

Time	IVIG group	Control group
Before	73.76 ± 27.7%	85.76 ± 8.6%
24 hours	93.22 ± 46.8%	97.9 ± 23%
7 days	88.52 ± 35%	94.1 ± 29.4%
14 days	88.1 ± 42.3%	113.4 ± 27.5%

n_1 (IVIG) =5

n_2 (control) = 5

Table 3.24 Serum opsonisation of GBS (mean ± SD) in IVIG and control groups during the third infusion period

Time	IVIG group	Control group
Before	76.8 ± 39.2%	93.9 ± 9%
24 hours	172.63 ±18.1%	80.15 ± 2.5%
7 days	67.84 ± 24.7%	65 ± 8%
14 days	89.1 ± 53%	65.75 ± 91%

n_1 (IVIG) =2

n_2 (control) = 2

Table 3.25 Serum opsonisation of GBS in IVIG group during the fourth infusion period (n =1)

Time	IVIG group
Before	51.6%
24 hours	37%
7 days	67.8%
14 days	48.7%

Table 3.26 Serum opsonisation of E.coli (mean ± SD) in IVIG group and control group during the first infusion period.

Time	IVIG group	Control group
Before	100%	100%
24 hours	173.74 ± 140.8%	106.27 ± 36.96%
7 days	148.88 ± 107.94%	114.71 ± 49.16%
14 days	187.31 ±148.1%	112.6 ± 64.66%

n_1 (IVIG) = 17
n_2 (control) = 17

Table 3.27 Serum opsonisation of E.coli (mean ± SD) in IVIG and control groups during the second infusion period.

Time	IVIG group	Control group
Before	134.5 ± 69.07%	104.32 ± 61.39%
24 hours	138.14 ± 98.51%	112.96 ± 55.9%
7 days	134.06 ± 104.23%	107.34 ± 51.96%
14 days	166 ± 147.63%	94.24 ± 58.91%

n_1 (IVIG) = 5
n_2 (control) =5

Table 3.28 Serum opsonisation of E.coli (mean ± SD) in IVIG and control groups during the third infusion period.

Time	IVIG group	Control group
Before	202.16 ± 191.96%	56.3 ± 21.21%
24 hours	223.86 ± 205%	46.85 ± 18.59%
7 days	218.7 ± 109.31%	41.8 ± 30.46%
14 days	186.4 ± 81.17%	61.6 ± 8.62%

n_1 (IVIG) = 2

n_2 (control) = 2

Table 3.29 Serum opsonisation of E. coli in IVIG group during the fourth infusion period (n =1)

Time	IVIG group
Before	243.8
24 hours	355.2
7 days	278
14 days	337

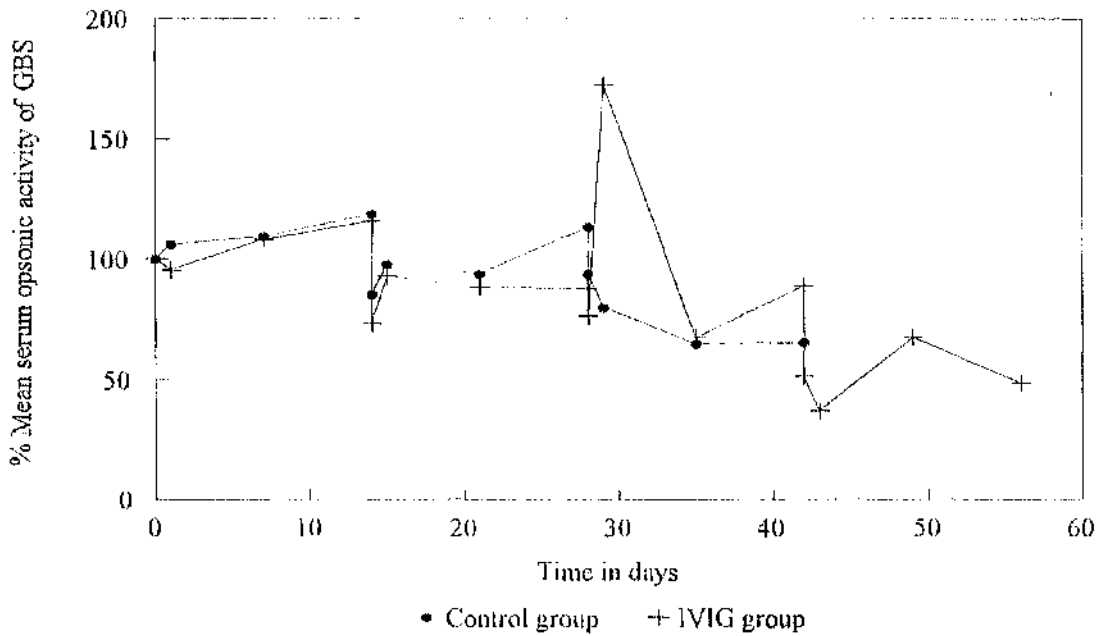

Figure 3.12 Mean serum opsonic activity of GBS in babies in the IVIG and control groups [n_1 (control) = 17 n_2 (IVIG) = 17]

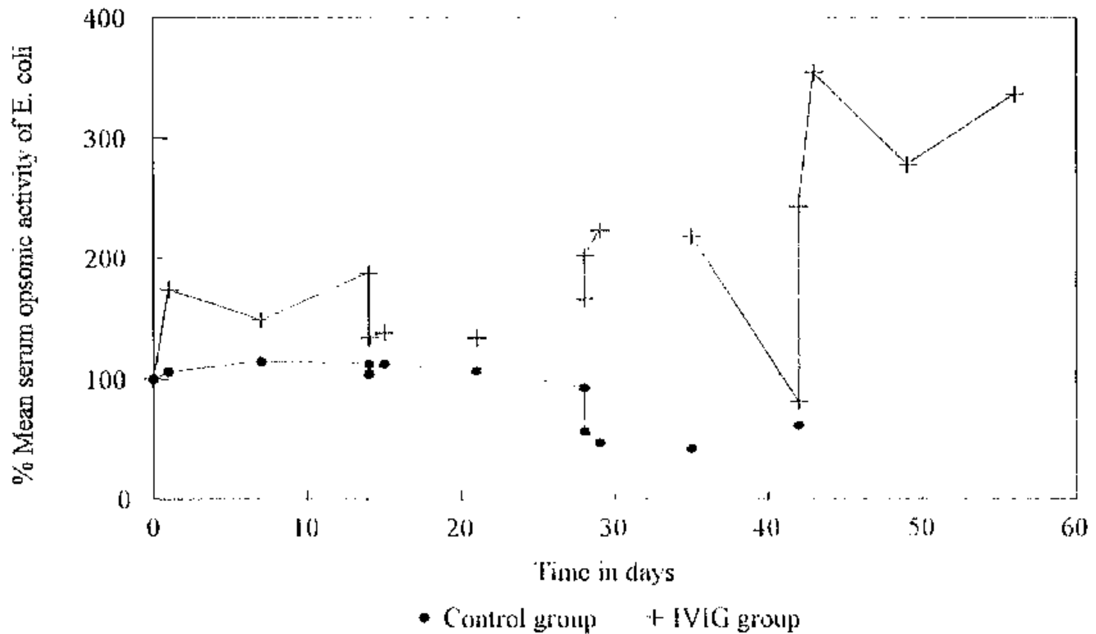

Figure 3.13 Mean serum opsonic activity of E. coli in babies in the IVIG and control groups [n_1 (control) = 17 n_2 (IVIG) = 17]

Table 3.30 Serum opsonic activity (mean ± SD) of E. coli in infected babies in the IVIG and control groups during the first infusion period

Time	IVIG group	Control group
Before	100%	100%
24 hours	137± 88.9%	108.5 ± 47.15%
7 days	136.32 ± 55.16%	112.38 ± 51.49%
14 days	136.7 ± 67.14%	122.36 ± 81.6%

n_1 (IVIG) = 5

n_2 (control) = 9

Table 3.31 Serum opsonic activity (mean ± SD) of E. coli in infected babies in the IVIG and control groups during the second infusion period

Time	IVIG group	Control group
Before	147.52 ± 72.32%	104.32 ± 61.39%
24 hours	144.65 ± 112.5%	124.45 ± 64.59%
7 days	148.42 ± 114.49%	107.34 ± 51.9%
14 dys	190.27 ± 158.53%	93.24 ± 58.91%

n_1 (IVIG) = 4

n_2 (control) = 5

Table 3.32 Serum opsonic activity (mean ± SD) of E. coli in infected babies in the IVIG and control groups during the third infusion period

Time	IVIG group	Control group
Before	252.3 ± 242.1%	56.3 ± 21.2%
24 hours	283 ± 251.16%	48.85 ± 18.5%
7 dys	218.7 ± 109.3%	41.8 ± 30.4%
14 dys	186.4 ± 81.1%	61.6 ± 80.6%

n_1 (IVIG) = 2

n_2 (control) = 2

Table 3.33 Serum opsonic activity (mean ± SD) of GBS in infected babies in the IVIG and control groups during the first infusion period

Time	IVIG group	Control group
Before	100%	100%
24 hours	91.36 ± 27.4%	101.6 ± 32.16%
7 days	100.28 ± 64.91%	110.27 ± 41.4%
14 days	72.72 ± 27.65%	124.82 ± 80.7%

n_1 (IVIG) = 5

n_2 (control) = 9

Table 3.34 Serum opsonic activity (mean ± SD) of GBS in infected babies in the IVIG and control groups during the second infusion period

Time	IVIG group	Control group
Before	73.22 ± 31.9%	85.76 ± 8.64%
24 hours	94.8 ± 53.96%	97.9 ± 23%
7 days	90.3 ± 40.2%	148.1 ± 148.4%
14 days	93.75 ± 46.63%	113.4 ± 27.48%

n_1 (IVIG) =4

n_2 (control) = 5

Table 3.35 Serum opsonic activity (mean ± SD) in infected babies in the IVIG and control groups during the third infusion period

Time	IVIG group	Control group
Before	71.2 ± 53.7%	93. ± 9.05%
24 hours	65.95 ± 49.56%	80.15 ± 2.87%
7 days	67.85 ± 24.67%	65 ± 8.05%
14 days	89.1 ± 53.03%	65.7 ± 9.2%

n_1 (IVIG) = 2

n_2 (control) = 2

Table 3.36 Serum opsonic activity of E. coli (mean ± SD) in non infected babies in the IVIG and control groups

Time	IVIG group	Control group
Before	100%	100%
24 hour	197.23 ± 166.87%	105.43 ± 27.19%
7 days	156.45 ± 129.2%	119.66 ± 48.16%
14 days	181.6 ± 171.27%	101.75 ± 40.99%

n_1 (IVIG) = 12
n_2 (control) = 8

Table 3.37 Serum opsonic activity of GBS (mean ± SD) in non infected babies in the IVIG and control groups

Time	IVIG group	Control group
Before	100%	100%
24 hour	96.06 ± 43.83%	113.3 ± 31.06%
7 days	105.37 ± 50.95%	110.87 ± 24.13%
14 days	128.2 ± 121%	111.80 ± 39.93%

n_1 (IVIG) = 12
n_2 (control) = 8

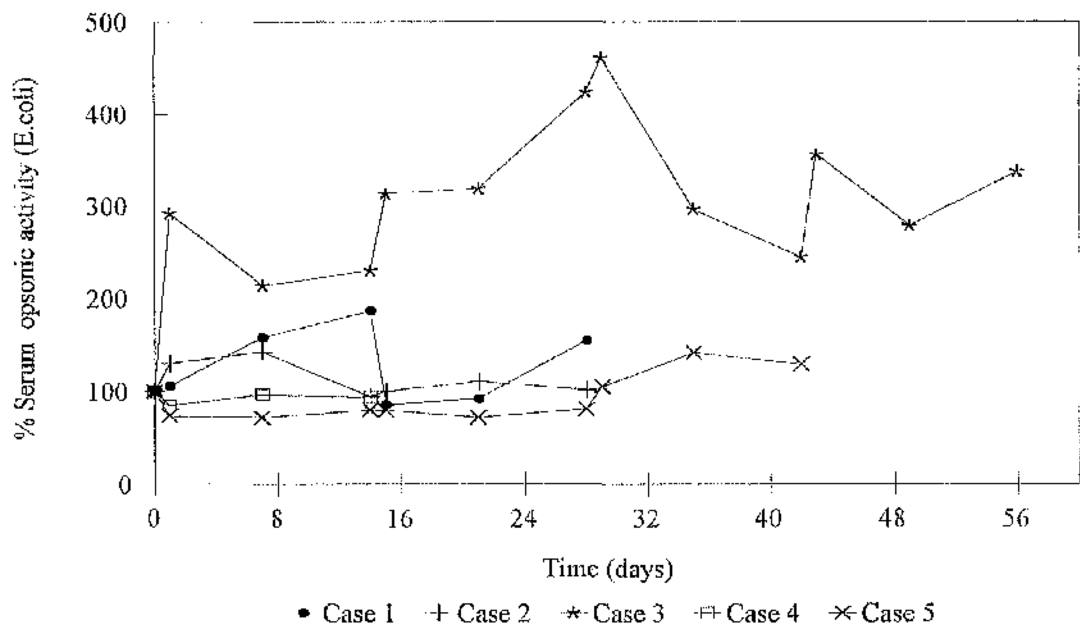

Figure 3.14 Serum opsonic activity of E. coli in infected babies in the IVIG group

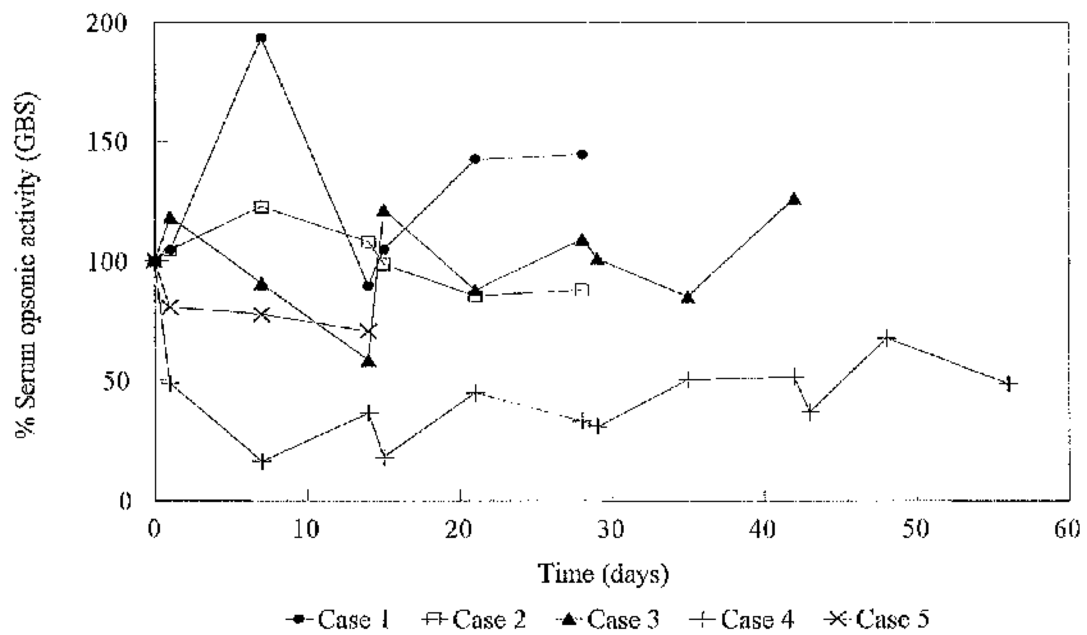

Figure 3.15 Serum opsonic activity of GBS in infected babies in the IVIG group

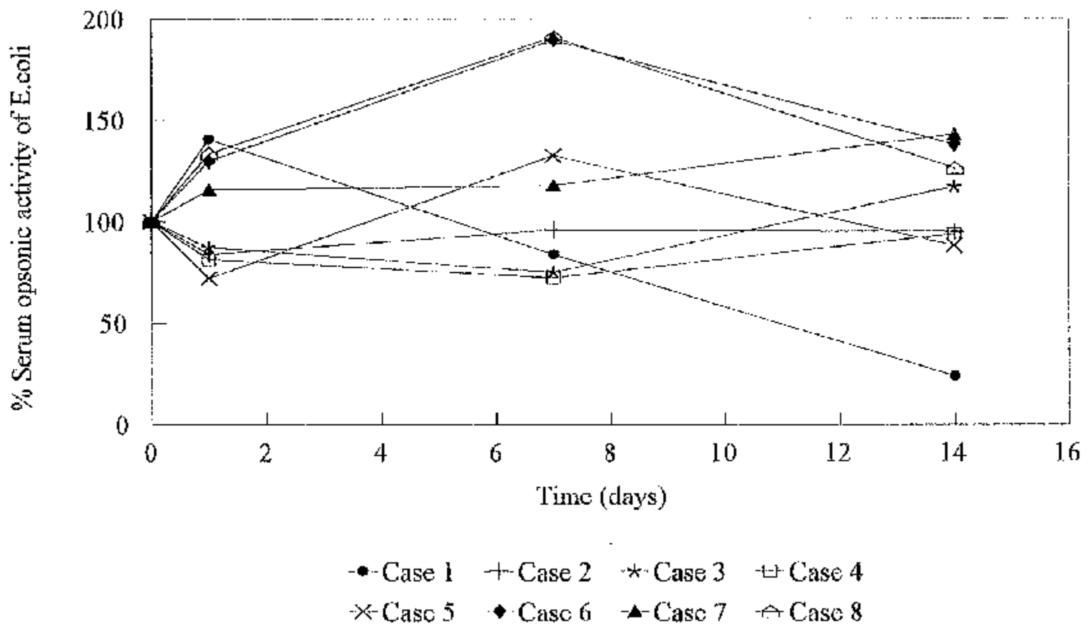

Figure 3.16 Serum opsonic activity of E.coli of serum from non infected babies in the control group

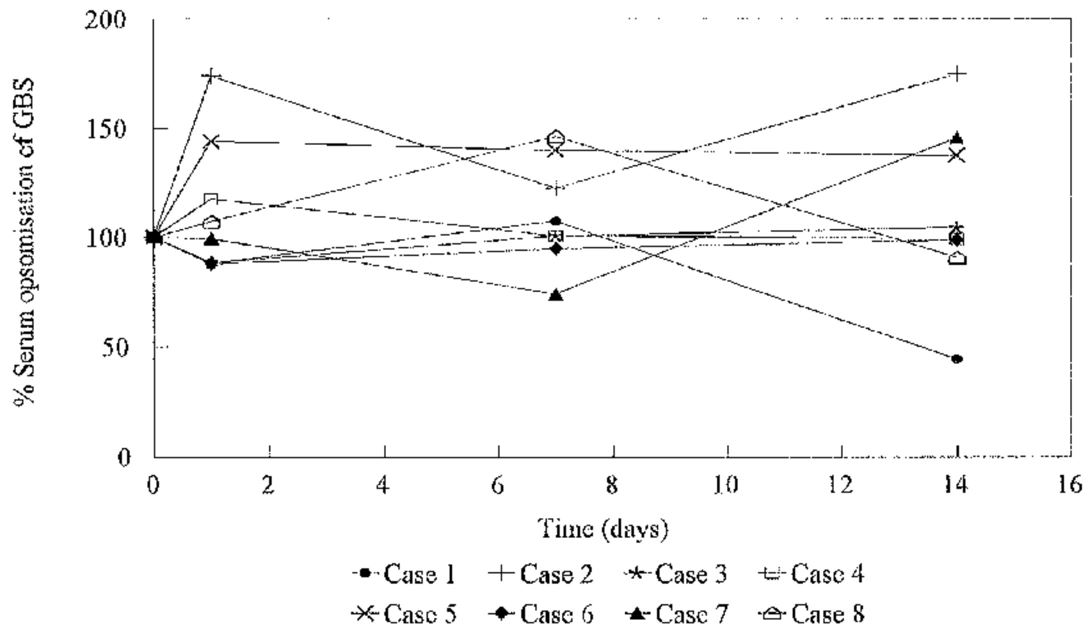

Figure 3.17 Serum opsonic activity of GBS of serum from non infected babies in the control group

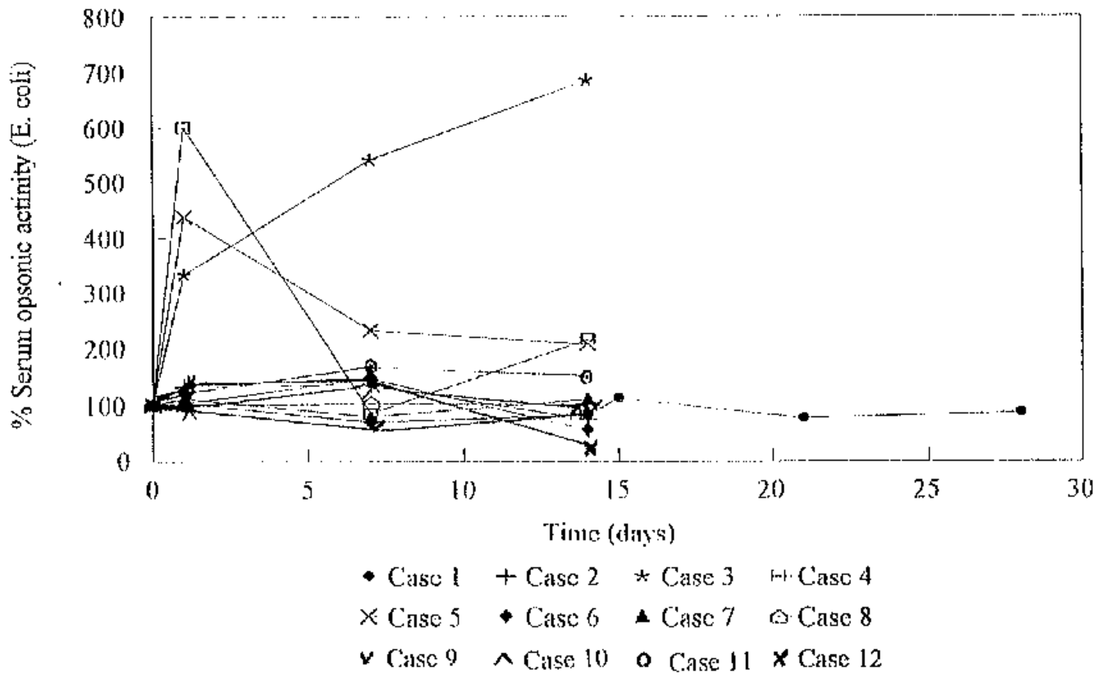

Figure 3.18 Serum opsonic activity of E. coli of non infected babies in the IVIG group

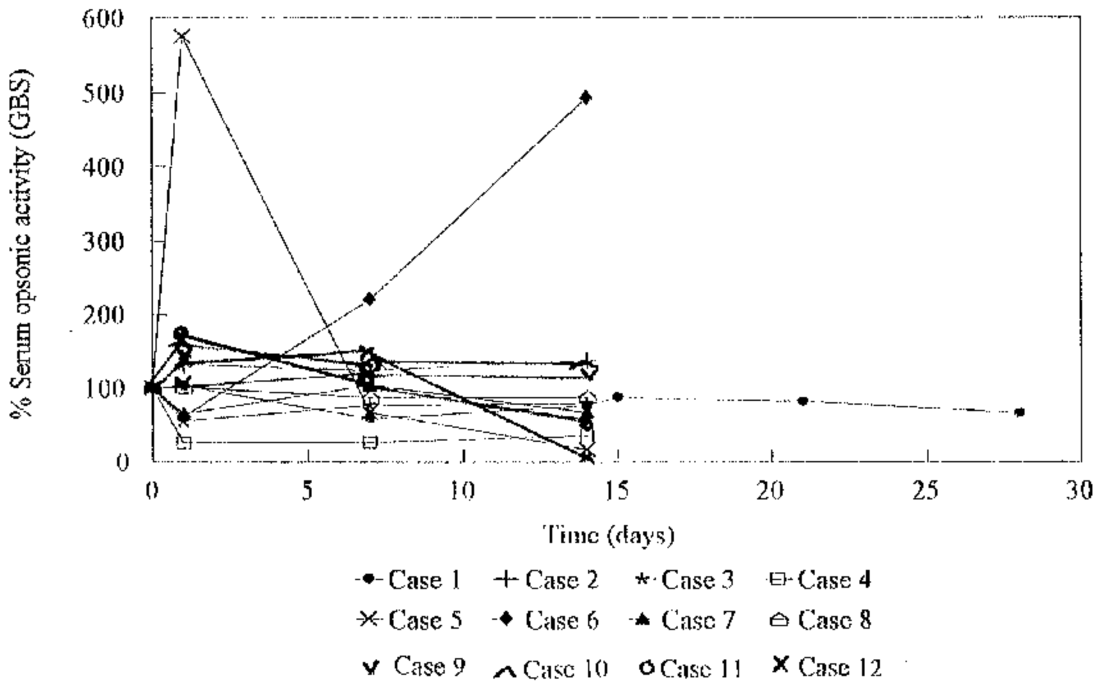

Figure 3.19 Serum opsinic activity of GBS of non infected babies in the IVIG group

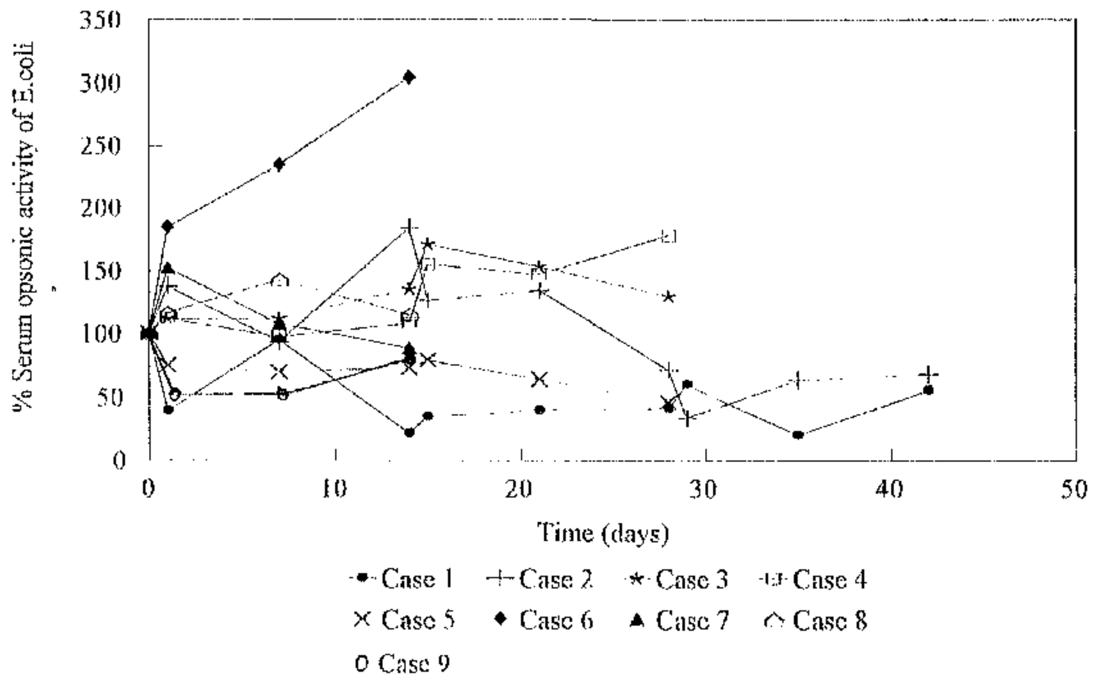

Figure 3.20 Serum opsonic activity of E. coli of infected babies in the control group

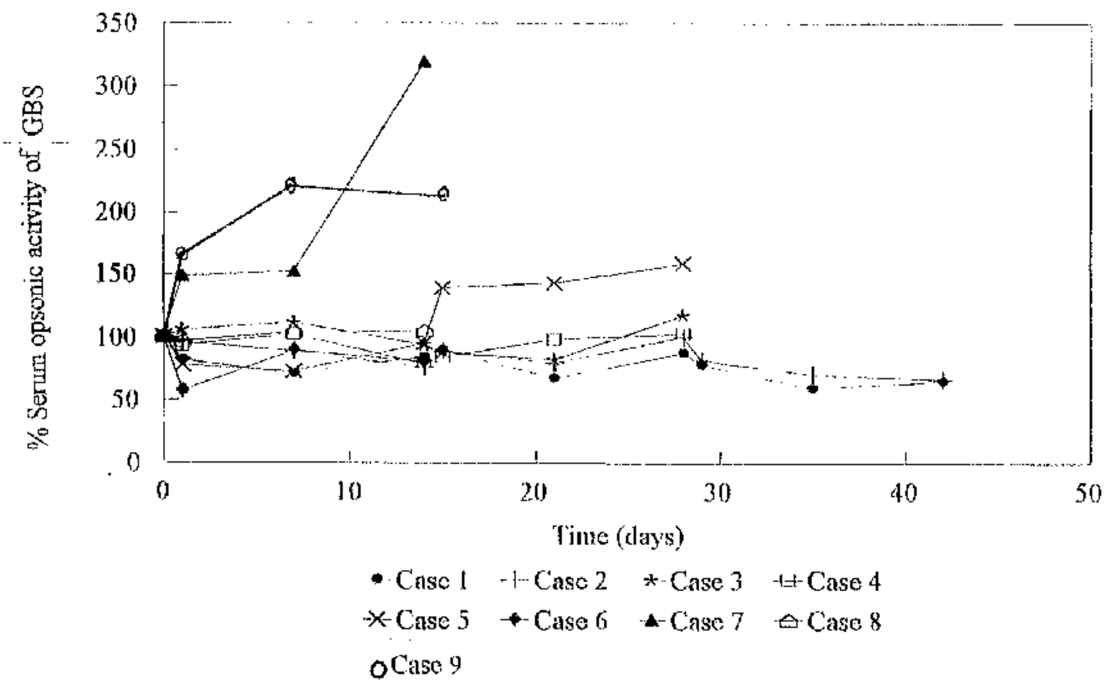

Figure 3.21 Serum opsonic activity of GBS of infected babies in the control group

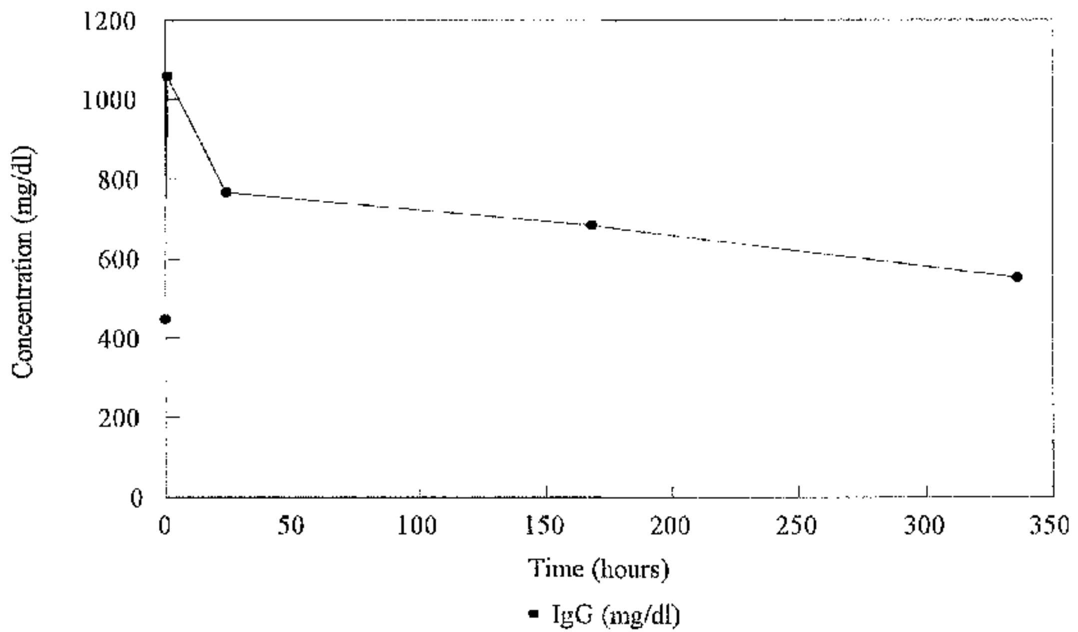

Figure 3.22 Mean serum IgG concentration in non-infected babies receiving the first infusion in the IVIG group
(n= 12)

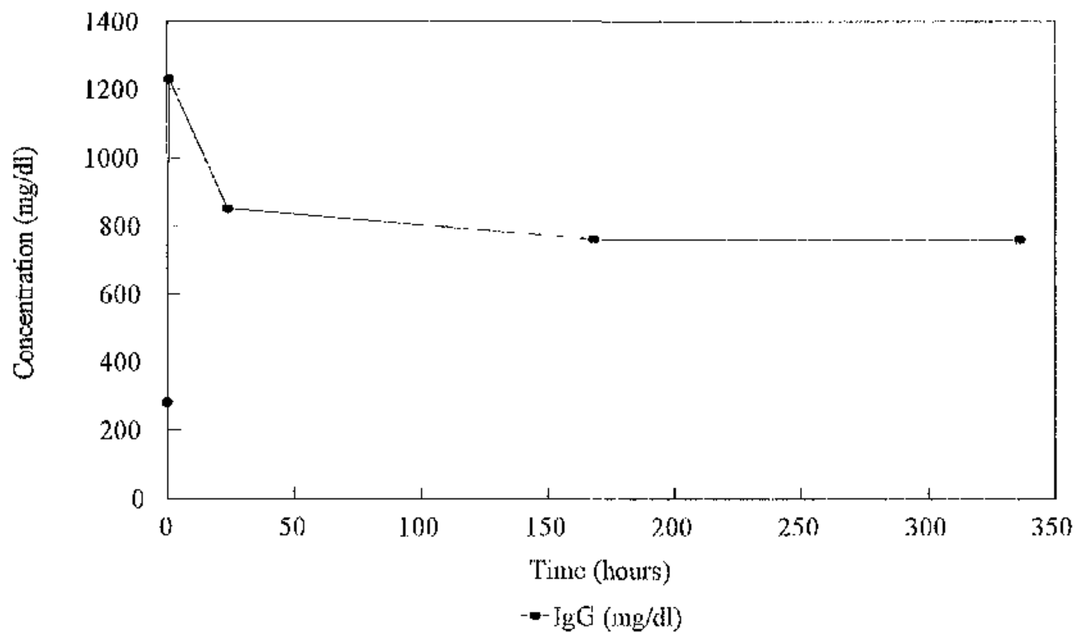

Figure 3.23 Mean serum IgG concentration in non-infected babies receiving the second infusion in the IVIG group
(n= 1)

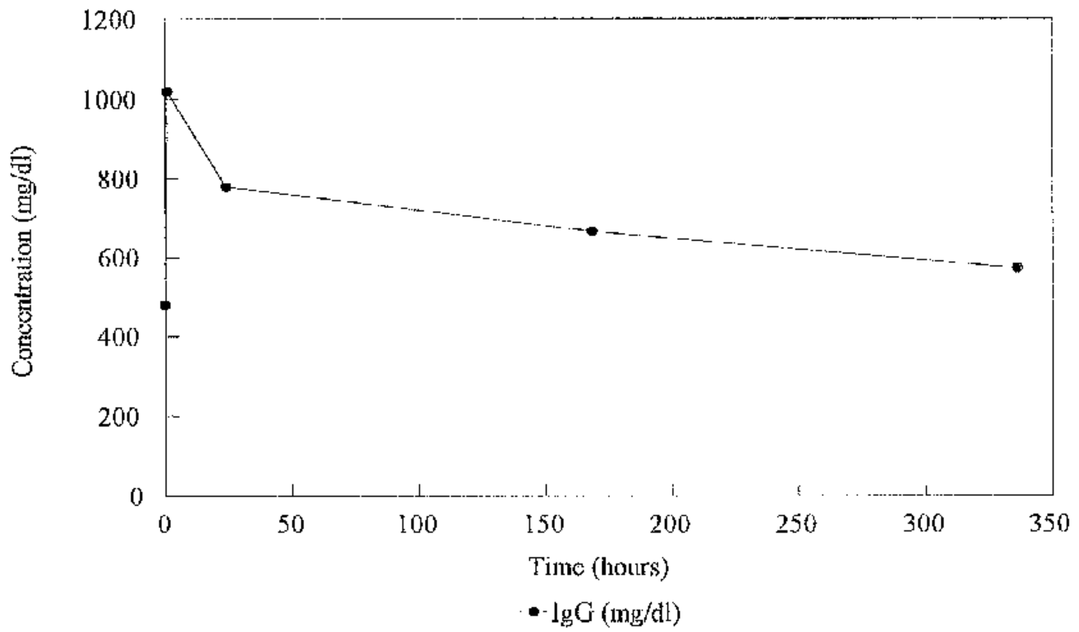

Figure 3.24 Mean serum IgG concentration in infected babies in the IVIG group before and after the first infusion
(n= 5)

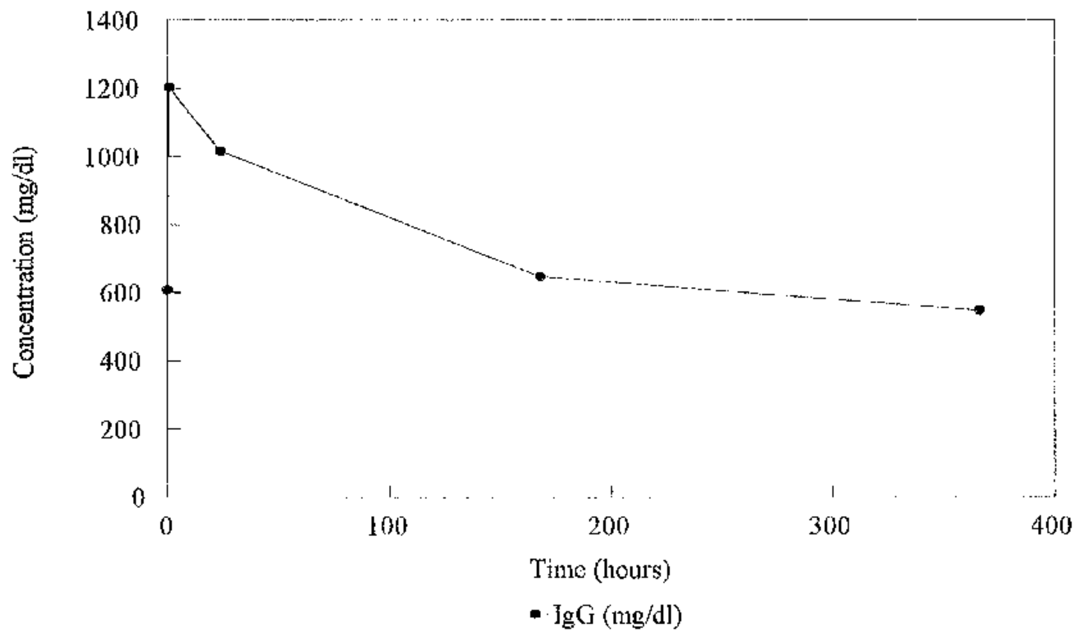

Figure 3.25 Mean serum IgG concentration in infected babies in the IVIG group before and after the second infusion
(n = 4)

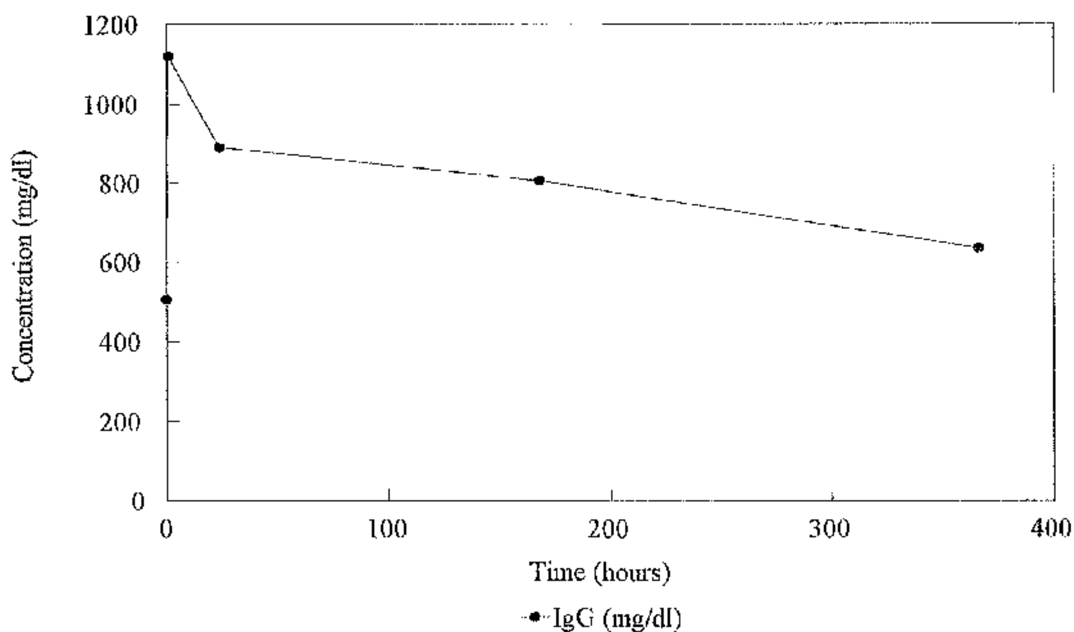

Figure 3.26 Mean serum IgG concentration in infected babies in the IVIG group before and after the third infusion
(n=2)

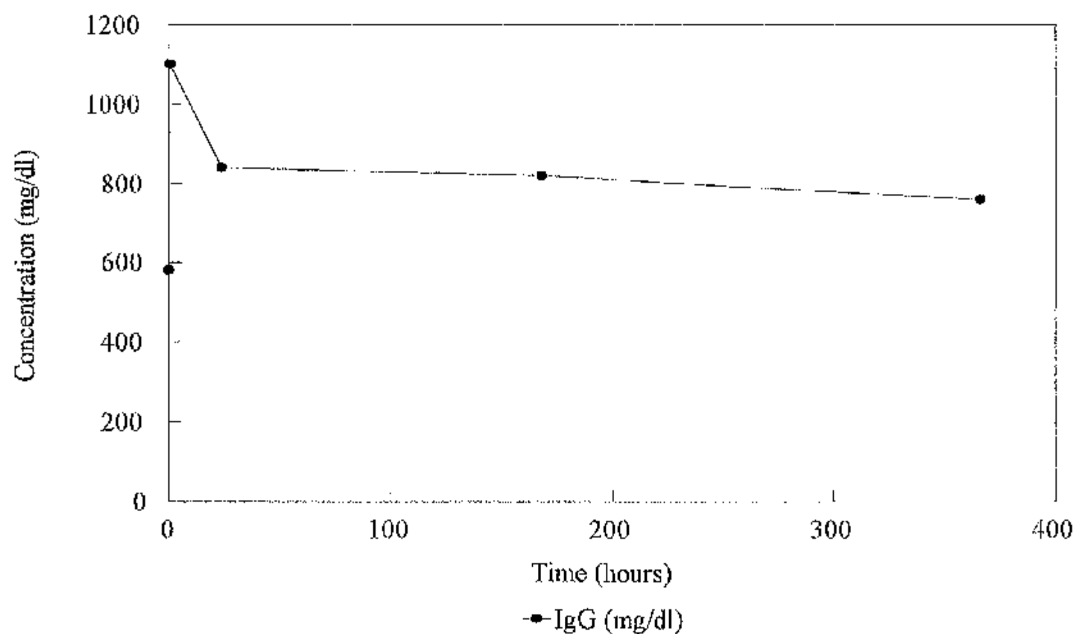

Figure 3.27 Mean serum IgG concentration in infected babies in the IVIG group before and after the fourth infusion
(n=1)

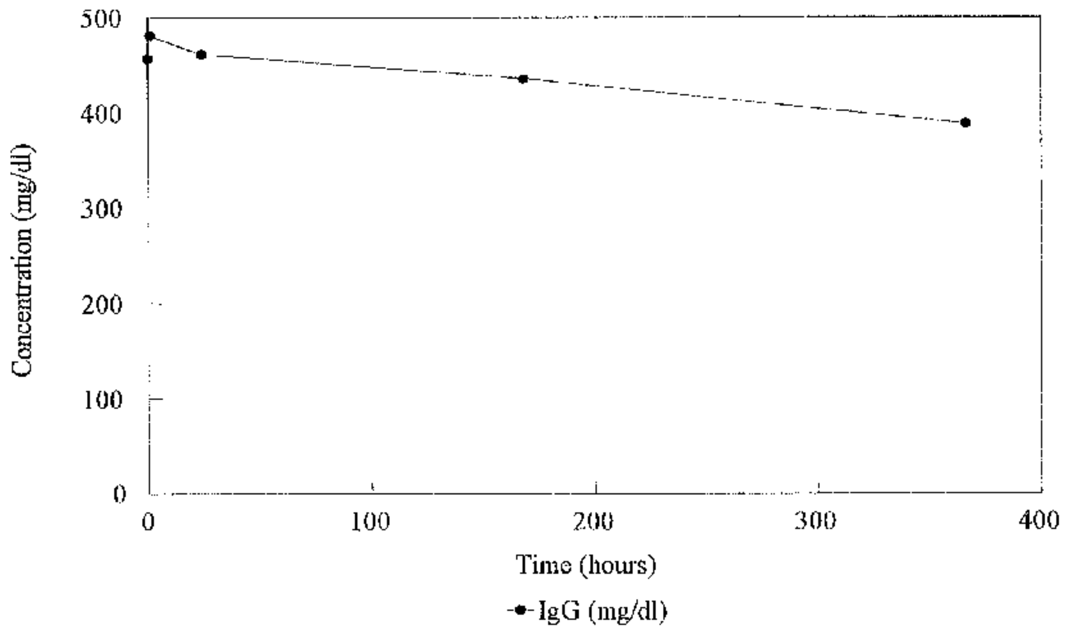

Figure 3.28 Mean serum IgG concentration in infected babies in the control group before and after the first infusion
(n=9)

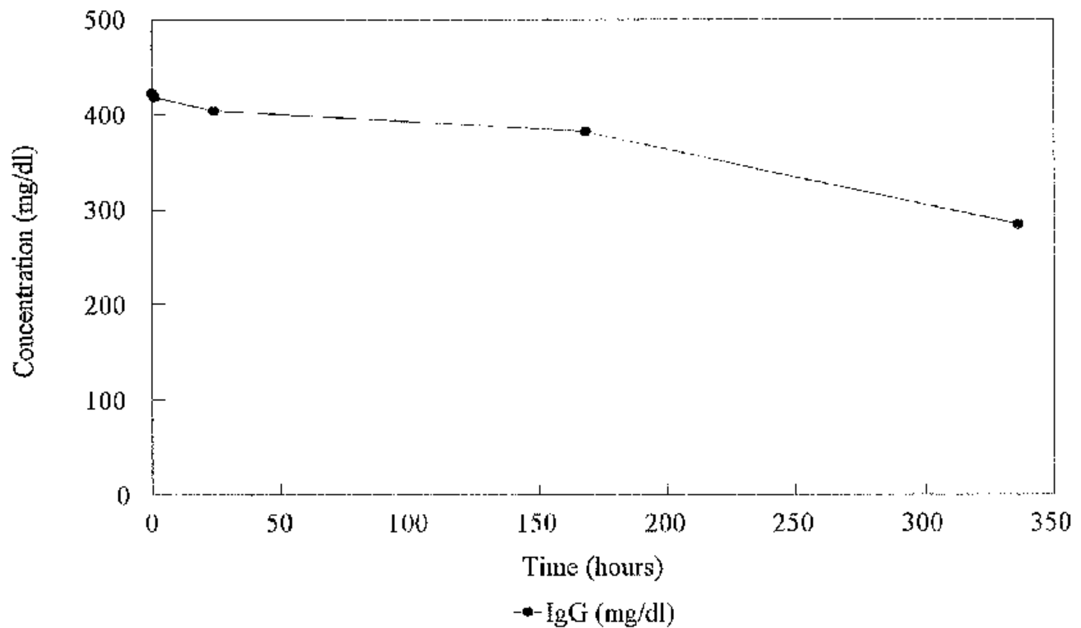

Figure 3.29 Mean serum IgG concentration in infected babies in the control group before and after the second infusion
(n= 5)

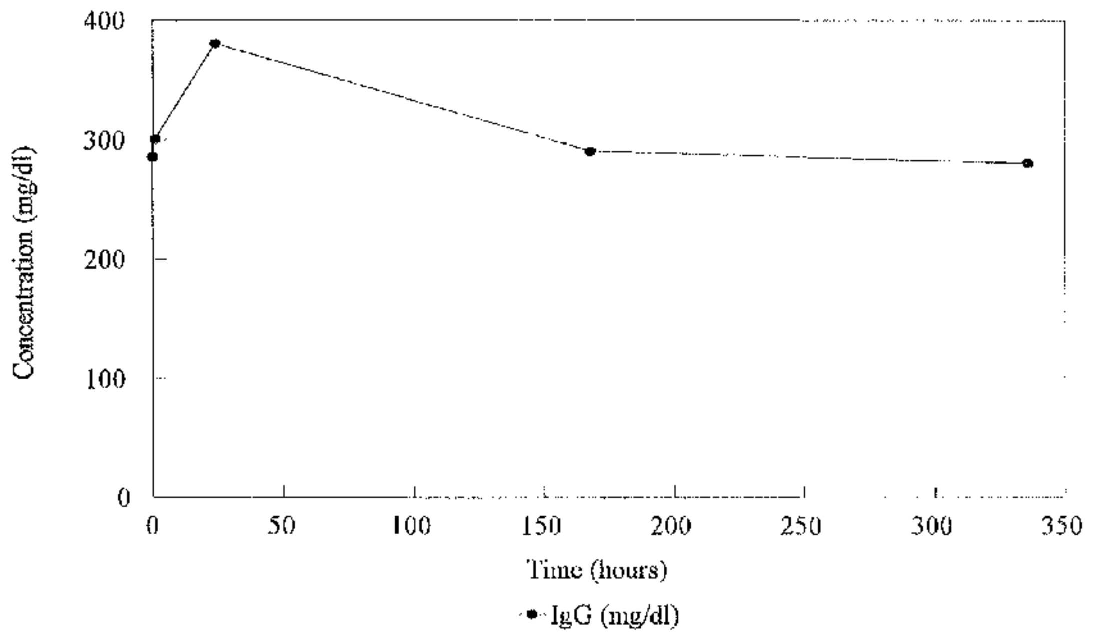

Figure 3.30 Mean serum IgG concentration in infected babies in the control group before and after the third infusion
(n= 2)

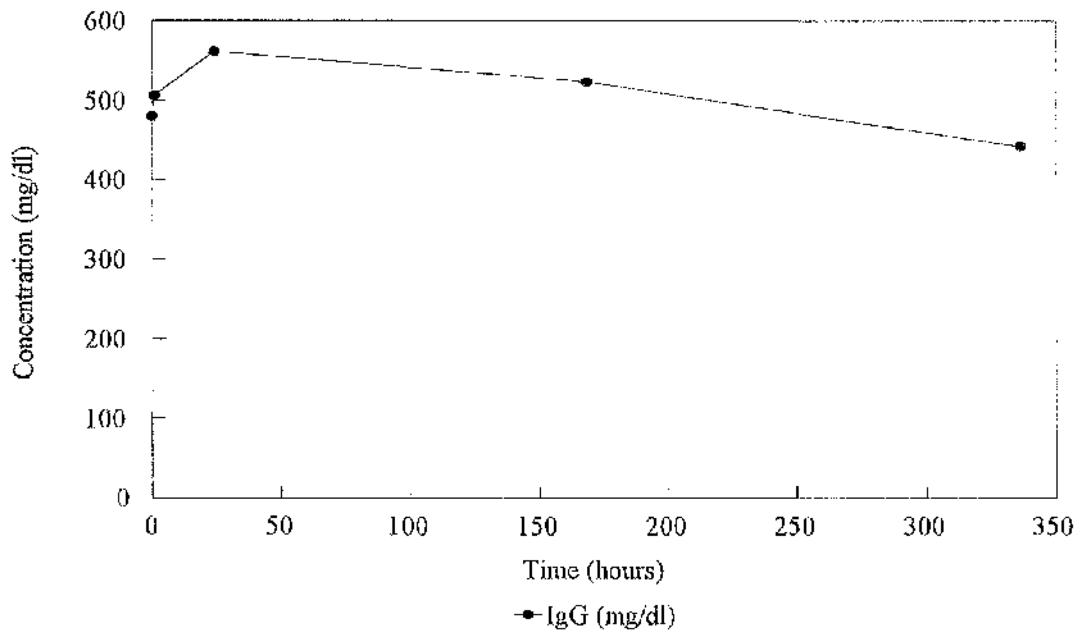

Figure 3.31 Mean serum IgG concentration in non-infected babies in the control group before and after the first infusion
(n = 8)

2.4 Side effects

In the treatment group there was no significant difference in HR, RR, BP at 30 minutes pre-infusion, 30 minute intervals during the infusion and 30 minutes post-infusion. No temperature instability ($\pm 1\ ^{o}C$) was noted. All of the infants were clinically stable at the time of the infusion. There were no significant changes in urine samples tested for blood and protein before, and 24 hours after the infusion. Serum glucose, electrolytes, osmolality, total bilirubin, blood urea, haemoglobin, WBC and platelets showed no significant difference before, during and after the IVIG infusion.

There was no significant difference in the mean haemoglobin concentration before the infusion and after 24 hours. Seven babies in the IVIG group had a decrease in the mean haemoglobin (14.17 ± 2.42 g/dl, range: 12.4 - 18.7) and haematocrit concentration 24 hours after the infusion but this decrease was not significantly different from the mean pre-infusion level (16.36 ± 2.04 g/dl, ranges: 14.1 - 20.5).

This decrease in the haemoglobin was also noted in the control group. Six babies showed a decrease in the mean haemoglobin from 16.7 ± 2.86 g/dl (range: 13.5 - 20.8) to 14.0 ± 2.4 g/dl (range: 11.6 - 17.5) post-infusion, $p > 0.05$. These results also shows no significant difference. This reduction in haemoglobin level may be due to blood sampling, hemodilution, low serum erythropoietin levels and the course of their illness.

In addition there was no significant difference in the duration of phototherapy between the two groups as there was no significant difference in the serum bilirubin levels.

Seven (7) out of seventeen (17) babies in the IVIG group (41.7%) developed IVH/PVH in comparison to ten (10) out of seventeen (17) babies in the control group (58.8%). This was not statistically significant. $\chi^2 = 0.750$, $0.10 < p < 0.50$.

There was no evidence of renal impairment in infants who received IVIG. There was no significant difference in the mean blood urea and serum creatinine concentration before the infusion and 24 hours, 7 days and 14 days post-infusion in the IVIG and control groups. There was an increase in blood urea in a number of babies in both groups. Four babies in the control group and two babies in IVIG group showed an increase in blood urea concentration during the 14 days post-infusion period (but no significant difference in blood urea between the pre-infusion level and 1,7and 14 days post-infusion, $p > 0.05$). During the period of increased blood urea, the babies were unwell, three babies in the control group had bronchopulmonary dysplasia and were being treated with dexamethasone which could have accounted for the rise in blood urea. An increase in blood urea may be due to the course of the illness rather than to any effect of IVIG given.

One baby in the treatment group developed nephrotic syndrome at the age of 120 days. This baby had received four doses of IVIG. Blood urea was increased to 9.2 mmol/dl at 33 days old and returned to normal level within 5 days. At that time she had infection episodes and suspected necrotising enterocolitis.

Chapter 4

DISCUSSION

4.1 Effect of IVIG in the prophylaxis of late onset sepsis

There are a variety of host defence abnormalities in neonates that help to explain the susceptibility of neonates to overwhelming bacterial infection. Many methods of treatment have been tried to correct the host defence abnormality in preterm babies. These include granulocyte transfusion, fibronectin and immunoglobulin transfusion. But the only method of treatment that gaves some satisfactory results in some studies was immunoglobulin infusion.

In different trials immunoglobulin was given in different protocols, preparations and doses, as well as to different study populations to prevent or to reduce the incidence of infection in neonates. These studies have focused on the VLBW infant in an attempt to correct the postnatal physiological and profound hypogamma-globulinaemia.

Most of the studies monitored nosocomial bacterial infection. The reported studies have employed different types of immunoglobulin preparations, different dose regimens, and a varied range of duration of IVIG therapy. Some studies provided IVIG during part but not during all of the potential at risk period for infection.

In some of these studies, the infection rate was reduced during the IVIG treatment period and increased during the period when the infants did not receive IVIG therapy.

Since the study in 1963 by Amer et al.[157] who noted a reduction in the incidence of death and non-lethal infection of premature neonates by administration of intramuscular injection of IgG at a dose of 3 ml/Kg every month, a number of subsequent studies were carried out using IVIG which is less painful to administer. These studies explored the relationship between the administration of IVIG and the prevention or treatment of neonatal infection. As noted in the review of literature, many trials did not convincingly answer the question of the efficacy of IVIG in the prophylaxis or treatment of bacterial infection.

Over all, some of these recent studies have demonstrated a beneficial effect of IVIG in reducing the incidence of nosocomial infection in VLBW infants, while others have not shown any statistical difference in the rate of infection.

These differences might be related to the dosing regimens, constitution of the IVIG preparation, sample size, definition of infection or the incidence of infection in the study population.

The aim of this study was to evaluate and compare the efficacy of IVIG on the prevention of sepsis in vulnerable VLBW infants who suffered from respiratory difficulty early in life, needed intensive life support and various procedures including intubation, umbilical venous and arterial catheterisation, insertion of long lines and drainage of pneumothoraces. All of these procedures are traumatic and carry with them an increased risk of infection.

Most studies reviewed administered IVIG to babies born prematurely and given according to their birth weight but did not specify whether this was limited to those needing intensive life support. Therefore the incidence of infection in their studies may

be low in the control and treatment groups in comparison to the incidence of sepsis in this study.

In our study IVIG was given to VLBW infants needing intensive care and aimed at preventing late onset infection. Data obtained from our results show no significant reduction in the incidence of proven sepsis in the IVIG treated group. Despite the observation of decreased incidence of proven sepsis in IVIG treated group (29.9%) in comparison to the control group (52.9%), this does not reach a statistically significant level.

The incidence of infection was more obvious in babies with birth weight ≤ 1000 grams (IVIG and control group) probably due to their prolong stay in intensive care and prolonged exposure to invasive techniques (e.g., mechanical ventilation, umbilical catheters, pleural drainage, total parenteral nutrition) to potential infectious agents, and a very under-developed immune mechanism, so that IVIG therapy alone may not alter the incidence of infection.

Because of the small number of babies included in our study, our study was not able to show any statistically significant effect of IVIG therapy in the prevention of infection in VLBW infants. This is consistent with the results obtained from many other studies[149,152,153,155].

The question still to be answered is whether IVIG has any role to play in the prevention of late onset sepsis in VLBW infants. From the results of our study, we are not able to answer this question. One point to be considered is the high incidence of proven sepsis in the control group in comparison to the IVIG treated group where both had similar clinical parameters, and both received the same treatment and intensive care management.

In this study the infection episodes occurred in the group of babies receiving IVIG when IgG levels were below 680 mg/dl. In the group which received 5% dextrose, infection occurred when IgG concentration was below 640 mg/dl. We did not measure the IgG level at the time of infection. Other studies demonstrated an increased incidence of infection among infants with serum IgG levels below 400 mg/dl[136].

Therefore the best result of IVIG administration may be obtained by maintaining the serum IgG concentration above the target level of 700 mg/dl (the lower limit of the normal IgG concentration in full term infants). This level can be achieved by frequent measurement and administration of IVIG to maintain the serum IgG level.

The reduction in the number of proven septic episodes in our study (within 14 days post-infusion) in the IVIG group was approximately 40% in comparison to the control group, even though this reduction did not reach the significant level. This is consistent with the result obtained by Baker et al.[146], who noticed a reduction in the incidence of proven sepsis of approximately 30% when IVIG was given during the first, and second weeks and at fortnightly intervals thereafter.

The infants in our study did not develop infections with organisms widely recognised as pathogens in neonates such as gram negative rods or group B streptococci. Rather, the infecting microflora represented organisms commonly encountered in nosocomial infection in many intensive care units. This included a high rate of Staphylococcus infection.

88.5% of infective episodes in both the IVIG and control groups were due to Staphylococcus epidermidis, 5.7% were due to Candida albicans, 2.9% were due to Enterobacter species and another 2.9% were due to Enterococcus faecalis. These

were nearly the same micro-organisms noted in other studies where most of the episodes of infection were caused by Staphylococcus epidermidis and S. aureus[151,154,155]. Weisman et al.[152] noted that two thirds of the infected babies in their study were infected with gram positive organisms, most commonly S. epidermidis.

It is the increased survival of these high risk infants which results in long stays in intensive care units resulting in increased exposure to microflora that leads to nosocomial infection with those micro-organisms. The development of infection with these organisms is usually associated with the use of indwelling catheters, respirators and other invasive procedures rather than to low antibody levels.

The most common cause of early onset neonatal sepsis in most neonatal units continues to be group B streptococci and E. coli. Both of these pathogens are encapsulated and the antibody plays an important role in effective phagocytosis and killing of these bacteria, although recent data suggest that there may be a role for the antibody in the killing of other pathogens such as S. epidermidis[158]. The role of the antibody against other neonatal pathogens such as Candida albicans and Enterococci remains to be determined. Therefore the role of IVIG therapy is less clear where these pathogens predominate as occurs in late onset sepsis.

It is unlikely, therefore that any IVIG focused on the prevention of infection caused by these organisms will ever show truly beneficial prophylactic effects.

88.2% of mothers in the control group as opposed to 52.9% of mothers in the IVIG group received ante-natal steroids. However none of the babies in the IVIG group and control group had infection during the first 5 days of life making it unlikely that any of those infections was due to maternal ante-natal steroid therapy. A previous

study[159] shows that prenatal and/or postnatal steroid treatment does not affect PMNs chemotactic maturation in preterm neonates.

The results of our study did not show any significant difference in suspected septic episodes between the two groups during the 14 day post infusion period. But the incidence of suspected sepsis after 14 days post-infusion was significantly increased in the IVIG group. This difference was mainly noted in babies with birth weight of 1001-1250 grams. This difference may be due to the development of a chest infection in three babies and high colonisation with different types of micro-organisms such as S. epidermidis, S. aureus, Enterobacter species, Enterococcus faecalis and Pseudomonas pyocyanea.

We noted an increase of suspected septic episodes in both groups. Some of these episodes may have been true infection episodes, as it is known the microbiologic cultivation techniques do not detect all episodes of infection. The frequent use of antibiotics in this population may affect the rate of recovery of pathogenic micro-organisms. Some of these infection episodes may have been due to viral infection which can not be detected by ordinary culture methods. For this reason, we examined the effect of IVIG on other parameters such as duration of antibiotic therapy, the duration of intensive life support and hospitalisation. We found that the administration of IVIG did not alter the duration of any of these parameters.

A better strategy in the future may be the use of hyperimmune intravenous immunoglobulin or human monoclonal antibody preparations aimed at treating rather than preventing a particular neonatal infection.

Some studies[160,161] suggest that low concentrations of complement may contribute to the development and severity of bacterial sepsis in neonates and may

restrict the efficacy of proposed adjunctive therapies, such as the administration of IVIG.

The failure of many studies to show any beneficial effects of IVIG in reducing the incidence of infection may be due to many factors. The antibodies administered may not have been effective against the infecting pathogens, non immunoglobulin mediated host defence impairment (e.g., complement, neutrophil, fibronectin) interfering with the impact of standard IVIG therapy, reduced availability of antibody at the infection site, failure to achieve a target level of serum IgG or lack of sufficient sample size to detect such deficiencies or other unidentified variables.

The results of our study did not show any significant effect of IVIG in the incidence of suspected necrotising enterocolitis. The role of immunoglobulin therapy for enteric diseases and necrotising enterocolitis is unclear. Several studies[155,162] have addressed the role of IVIG in preventing nosocomial infection and necrotising enterocolitis in LBW infants and full-term infants, but only one[162] of them addressed gastrointestinal illness in full term and premature infants. It shows that administration of IVIG had no substantial effect. More specific therapy with oral immunoglobulin preparation has been suggested to prevent NEC. Eibl and co-workers[163] performed a randomised clinical trial using an oral immunoglobulin preparation (37% IgA and 26% IgG) in the prevention of NEC in infants weighing between 800 and 2000 grams at birth. No cases of NEC occurred among 88 infants receiving the oral IgA-IgG compared to 6 cases of NEC among 91 control infants. In our study we did not see any effect of breast milk in the prevention of systemic infection or sign of suspected necrotising enterocolitis

In our study only 34.2% of proven septic episodes were accompanied by a raised C- reactive protein level. Two episodes were due to Candida septicaemia and

10 episodes were due to S. epidermides. This could be due to the fact that some infants seem to be incapable of mounting an appropriate acute-phase response[164,165]. Also it could be due to different organisms stimulating different patterns of acute-phase response. It has been shown that E. coli gives the most reliable increase in C-reactive protein[166], whereas in Sann's study[165] early onset group B streptococcal infection was less reliable.

In Sann's study, the serum C- reactive protein concentration was normal in 27% of patients with septicaemia. This was higher than that found by Sabel et al[167].

C- reactive protein is an acute phase protein synthesised by the liver within 6-8 hours of an inflammatory stimulus. It is produced most rapidly in response to bacterial infection. Although this response is non-specific, it is thought to give indirect evidence of infection.

Therefore in some neonatal units C- reactive protein is used in conjunction with good clinical assessment for the early diagnosis of infection, while the results of blood cultures are awaited. Serum C- reactive protein reflects the immediate effect of the treatment, therefore serial C- reactive protein measurements have been used for monitoring the efficacy of antibiotic treatment and to detect any recurrence of infection.

4.2 Effect of IVIG on serum IgG concentration

In babies $\leq$ 1000 grams birth weight the dose of 700 mg/kg of IVIG raises the mean serum IgG concentration one hour after the infusion to 280% of the pre-infusion level. The dose of 500 mg/kg of IgG increased the mean serum IgG

concentration by 215% in babies weighing 1001 to 1250 grams and by 185% in babies weighing 1251 to 1500 grams.

The increase of IgG concentration was affected by the post-natal age. The dose of 700 mg/kg given to babies with birth weight $\leq$ 1000 grams two days after birth increased the mean IgG concentration to 280% from the pre-infusion level,while the same dose given after two weeks increased the mean serum concentration to 226% of the pre-infusion level. At one month of age when the third dose was given the level peaked at 205% of the pre infusion level. The fourth dose was given at 6 weeks of age and increased the mean serum IgG concentration to 189% of the pre-infusion level.

The pharmacology of IVIG fits into a two phase disappearance curve. The first phase is short, beginning immediately after the infusion is administered. It lasts 1 to 4 days and may represent the redistribution of IgG between intravascular and extravascular compartments and clearance of denatured or aggregated IgG. In our study the serum IgG concentration declined by 19% to 28% from the peak post-infusion level 24 hours after an infusion. This was in keeping with observations made in other studies[111,116] where the IgG declined by 20% to 30% 24 hours post-infusion. We measured IgG on day 7 post-infusion and the level declined by 26% to 45% of peak post-infusion level.

This observation was noted by Noya and colleagues[112,113] who reported a 40% decline of serum IgG level within one day of an infusion among very low birth weight infants. The serum IgG level declined to 50% of post-infusion level within 3 to 5 days.

The second component is the half life and represents the catabolism of IgG which is linear and may be a better determinant of the required dose and dosing

interval to maintain the serum IgG at a therapeutic level. The half life of serum IgG in neonates is quite variable and difficult to predict[114]. The half life is dependent on gestation age, post-natal age, the presence or absence of infection, the level of total serum IgG, size of the extravascular space, functional activity of the IVIG and catabolic rate.

In neonates the short half life of IgG of about 11 to 14 days (4 to 7 days for a specific antibody) may result in part from an increased catabolic rate of administered gammaglobulin, consumption of antibodies by frequent neonatal infection or loss of protein in infants with renal or intestinal disease.

In this study the serum level of IgG measured 14 days post-infusion declined by 32% to 51% of the peak post-infusion level. This decline (after the first infusion) varied slightly between neonates of different birth weight. For instance it declined to 53% of the peak post-infusion level in infants with birth weight $\leq$ 1000 grams and to 60% in infants with birth weight 1251 to 1500 grams.

A significant variability was observed among these individual neonates with respect to the half life of the infused IVIG which depended on several factors (discussed before) and on the peak serum IgG concentration.

According to recent data[114,136] it is important to achieve and maintain the target level of serum IgG of 700 mg/dl (this level was chosen as the lower limit of the normal range of serum IgG in the cord blood among term infants) during the first month of life which is the high risk period for infection secondary to the wide use of invasive procedures.

Among neonates in our study with birth weight ≤1000 grams a dose of 700 mg/kg every two weeks did not sustain a target level of 700 mg/dl for 7 days in 69% of infusions and for 14 days in 77% of infusions. Also a dose of 500 mg/kg every two weeks given to infants with birth weight of 1001 to 1500 grams failed to sustain the target level for 7 days in 58% of infusions and for 14 days in 91% of infusions. Similar results were observed by Kyllonen et al.[114] in which a target level of 700 mg/dl was achieved in 90% of infusions performed in neonates weighing >1000 grams with a dose of 700 mg/kg. Tsakalidou et al.[140] also attained similar concentrations of at least 10 days duration in 81% of the infusions of 500 mg/kg.

Noya and colleagues[112] demonstrated a rapid decline of serum IgG concentration after one dose of IVIG to VLBW infants. The serum IgG concentration declined below 700 mg/dl 7 days after a dose of 500 mg/kg among 2 to 3 day old 1100 gram infants, but a dose of 1000 mg/kg was able to sustain this target level.

The small, less mature infants had an increased IgG half life with increased post-natal age which suggests the need for a shortened dosing interval in the first few weeks of life. The change in IgG elimination rate with time appears to be related to maturity.

Among neonates with birth weight of less than 1000 grams a dose as high as 900 mg/kg every two weeks did not sustain a target level of 700 mg/dl for 14 days in 60% of infusions. Even among infants weighing more than 1000 grams, it was difficult to sustain serum levels for two weeks with a dose of 500 mg to 900 mg/kg of IVIG[114].

These findings suggest that although administration of IVIG at conventional doses may result in a satisfactory increase of the total serum IgG levels in neonates

with birth weight > 1000 grams, it may not result in an analogous increase of all IgG subclass levels.

Specific antibodies belong to certain IgG subclasses. Antibodies against bacterial polysaccharide antigens are contained mainly in IgG2 and IgG4[168,169]. One could hypothesise that it may be desirable to achieve and maintain a target serum level of not only total IgG but also IgG subclasses.

Frequent monitoring of serum IgG levels will be necessary if the target level of IgG is to be maintained because of the wide range of IVIG kinetics in VLBW infants.

For the optimal use of IVIG, the dose given to preterm babies may need to be individualised on the basis of the serum IgG level drawn 48 to 72 hours after the initial infusion when the infused IgG has equilibrated into the extravascular compartment. This level needs to be checked again 7 days later if the target level is to be maintained.

Results of our study show that infection episodes developed when serum IgG was as high as 680 mg/dl, but another study[136] demonstrated infection episodes when IgG was at less than 400 mg/dl.

The level of IgG in premature infants is determined by the amount of maternal IgG transferred across the placenta, by the catabolism of maternal IgG and by the rate of synthesis of the infant's own immunoglobulin. This rate of synthesis is minimal in premature infants. In the blood of the preterm infants, the IgG concentration is low at birth and declines rapidly after. Therefore the group of babies receiving 5% dextrose represent the normal level of serum IgG in preterm babies of different birth weights and post-natal ages. The mean IgG concentration at birth in babies with birth weight ≤

1500 grams was 467 ± 137 mg/dl (ranges: 240-710 mg/dl) with wide variability among neonates. This is in keeping with Backers study[151]. Another study showed a reduced IgG concentration of under 200 mg/dl within a few weeks after birth.

The mean concentration of IgG in the control group at different postnatal ages was shown in Table 3.17. This is equivalent to the normal IgG concentration in premature infants at different gestational age (Table1.1).

These findings are nearly the same as the IgG level in babies born prematurely at 29 to 33 weeks gestation in the study of Ballow et al.[170], who found the mean IgG concentration was 368 mg/dl at birth, declined to 104 mg/dl at 3 months of age, and then slowly increased. Ballow and coworkers also found the mean concentration of IgG was increased only to 280 mg/dl at 10 months of age.

In another study[131], infants with birth weight of <1000 grams had serum IgG levels at birth of 489 ± 81 mg/dl and that they declined to 130 ± 34 mg/dl by 10 weeks of age, whereas those of >1000 grams had mean cord blood IgG level of 736 ± 47 mg/dl which declined to 418 ± 34 mg/dl by the first month of life. Another study[127] demonstrated markedly low serum IgG levels among small infants born between 25 to 28 weeks, with a serum IgG level as low as 60 mg/dl at 3 months of age. These lower serum levels may be associated with a high risk of infection.

In our study, even one dose of IVIG could maintain the serum IgG concentration at a higher level than in those who did not receive any IVIG (Table 3.20). One dose of IVIG kept the IgG concentration at age of one month at 130% higher than in the control group, 157% higher at 2 months of age and at 200% higher at 3 months. These higher levels were significant at ages 2 and 3 months but not significant at the age of one month.

In our study 29% of infants had a serum IgG level less than 200 mg/dl at the age of one month. 50% had a serum level of less than 200 mg/dl at two months and 90% had a serum level of less than 200 mg/dl at the end of the third month.

These results indicate an increased susceptibility of preterm babies to infection during the first 3 months of life especially during the first month when most invasive procedures are carried out.

4.3 Serum opsonic activity of E. coli and GBS

As we noted from the results of our study there was no significant effect of IVIG on serum opsonic activity for GBS and E. coli. This may be due to several factors including deficiency of complement as the serum was heated for 30 minutes at 56 ^{0}C to remove the effect of the complement, the IVIG used had decreased quantity of intact IgG, or the IVIG had a significant lot-to-lot variability in the concentration of functional type specific antibody.

Also there was no correlation between the time of infection episodes, serum opsonic capacity for E. coli and GBS, and the IgG concentration. There were no infection episodes when IgG concentration was more than 680 mg/dl.

The high peak values of E. coli opsonisation in some babies in the IVIG group were higher than those in the control group, and even the mean of the high peak value of E. coli opsonisation was higher than the mean in the control group but this did not reach a statistically significant level. This observation may be due to a high concentration of specific anti-E. coli antibody in the IVIG used.

However the serum opsonic capacity for GBS was not different in the IVIG group from that in the control group. One explanation might be due to a low concentration of specific anti-GBS antibody in the IVIG used, or to deficient complement levels leading to deficient opsonisation.

Opsonisation is the process by which bacteria are prepared for ingestion and intracellular killing by phagocytes such as neutrophils. Usually neutrophils do not recognise or ingest pathogenic bacteria unless the organisms are opsonised by the deposition of antibodies and/or complement proteins onto the bacterial surface. Many encapsulated bacteria such as type III group B streptococci and E. coli that express the K antigen resist activation of complement by the alternative pathway[171,172]. Moreover in the absence of a complement source, these organisms are not efficiently opsonised by the deposition of an antibody alone[172,173].

The organisms are ingested and killed efficiently by neutrophils after the surface deposition of both antibodies and complement proteins, especially degradation products of C3. Antibodies such as those that comprise IVIG must work in concert with the complement systems and neutrophils to achieve efficient opsonisation and bacterial killing.

In the serum of the preterm and ill full term baby, opsonisation was inefficient[174,175]. Although antibody deficiency may have accounted for this observation, it is possible that diminished complement concentration and activity may restrict the opsonic capacity of neonatal serum. For example, the concentration of various complement proteins have been noted to be diminished in the serum of septic neonates compared with those of healthy neonates. Death in septic neonates has been associated with decreased complement activity in the sera of the non-survivors[160].

The capacity of septic neonates serum to deposit C3 onto pathogenic bacteria was diminished and was not consistently enhanced by the IVIG infusion[161].

Marked variations were observed in the CL response with different bacteria and PMN from different normal donors. The variation among organisms may reflect activation of different metabolic pathways by various bacteria and the generation of different types and/or amounts of oxidative species. These are most likely to contribute to variations in CL among bacteria. Additionally, the extent of opsonisation were represented by both quantitative and qualitative differences in the type and/or the amount of serum opsonins like complement components or immunoglobulins coating the bacteria. The direct relationship between the amount of serum per bacterium and the amount of CL certainly suggests a cretical role for serum opsonins on the kinetics of CL.

The large range in the CL response among PMNs from different donors also suggests basic differences in the PMNs metabolic activity of normal people. These cellular differences could reflect variations in recognition, ingestion, enzymes, or oxidative species and enzymes released during phagocytosis.

Luminol-dependent CL is useful in determining serum opsonic activity because it is dependent on heat labile factors, such as complement, and correlates with the amount of serum per organism used for pre-opsonisation.

88% of infection episodes in this study were due to S. epidermidis. Therefore either there was a low concentration of anti-S. epidermidis antibody in the IVIG preparation used in this study or the anti-S. epidermidis antibody concentration was adequate but the antibody was not effective against this micro-organism. Recent data suggest there may be a role for the antibody in the killing of pathogens such as S.

epidermidis[158], and that there is an important role of the antibody in the infective phagocytosis and killing of E. coli and GBS. However the role of the antibody against other neonatal pathogens such as Candida albicans and enterococci is not known.

In this study 66.6% of S. epidermidis infection episodes were associated with the presence of a central intravenous long line. The most important factors affecting the incidence of infection are prematurity, as 88% of infection episodes occurred in babies with birth weight ≤ 1000 grams and the presence of a central intravenous long line. The group of babies with birth weight ≤ 1000 grams needed a prolonged period of intensive life support, more intensive care procedures such as intubation, UAC, UVC, central intravenous lines and drainage of pneumothoraces than those with birth weight ≥ 1000 gram.

4.4 Side effects

We found that the infusion of 500 mg/kg and 700 mg/kg of IVIG to preterm infants with birth weight of ≤ 1000 grams and 1001 to 1500 grams respectively was safe. There were no instances of hypersensitivity, inflammatory or toxic reactions as indicated by changes in the physiological or laboratory parameters.

These results support the observation from previous reports that neonates generally tolerate the infusion of IVIG quite well[114,133,141,148]. These are consistent with previous reports that adverse reaction to any transfused blood products in neonates is rare, in the order of 0.1%[133].

Our sample size was small and we would have observed only complications occurring with a high frequency. The incidence of suspected complications of administering intravenous immunoglobulin to neonates has been ≤ 0.05%[138].

In this study the incidence of IVH/PVH was more in babies receiving 5% dextrose than those receiving IVIG, but in both groups the incidence was high, which may have been due to prematurity and severity of respiratory distress syndrome. The babies included in the study were all ventilated and required extensive life support. This was contrary to the results of other studies[136] where there is an increase in the incidence of IVH/PVH among infants receiving IVIG compared to those who did not receive any treatment. This finding may be related to the osmolality and the volume of IVIG infused or to the relatively better over all condition of the non assigned group. Some previous data indicate an association between IVH/PVH and hypertonic infusions when these are infused rapidly[137,176].

In this study no significant increase of blood urea levels was noted in babies 24 hours post infusion of IVIG. Two babies showed an increase in blood urea level during the14 day period post-infusion but this may have been due to the course of their illness rather than due to IVIG.

One baby who received 4 doses of IVIG developed nephrotic syndrome at the age of 120 days, the last infusion have being given at the age of 46 days. The clinical picture at the time of presentation suggested renal vein thrombosis as a cause. The baby needed repeated doses of albumin and immunoglobulin. She also developed septicaemia with gram negative organisms at that time. The baby improved after a period of one month.

There have been no reports of the development of renal toxicity in neonates receiving IVIG. One study[168] showed a transient increase in blood urea immediately after the infusion which persisted for about 6 hours after the infusion. This may be related to the catabolism of the administered protein and perhaps not to impaired renal function.

In adults IVIG is generally well tolerated but there have been a number of reports[177,178] both of acute deterioration in renal function and pulmonary toxicity following IVIG infusion.

The cause of renal toxicity is not clear but it may be that high doses of IVIG result in an increased plasma and blood viscosity[132] which may affect capillary blood flow[131]. The increase in plasma viscosity may be due to the large amounts of sugar used as a stabiliser. This may explain why the renal toxicity is generally of limited duration and is self limiting.

Chapter 5

Conclusion

The ultimate goal in the management of neonatal infection is to try prevent its occurrence. The most direct way to reduce the incidence of this frequently devastating disease is to prevent premature birth.

Development of effective therapeutic intervention to prevent neonatal infection was tried before through studying the immune system and several preventative approaches have been used.

However, some of these treatments have been focused on the use of IVIG in premature babies with birth weight $\leq$ 1500 grams or less than 32 weeks of gestation.

From this study, we are not able to answer the question whether IVIG is useful in preventing infection in preterm babies. This may be due to the small number of preterm babies included. Although there is a high incidence of proven infection in the control group as opposed to the treatment group in babies $\leq$ 1000 grams birth weight, this did not reach statistical significance.

The administration of IVIG may not be effective in this and other big multicentre studies due to several factors.

1. The administered IVIG may have inadequate titers or lack of functional antibodies against the most prevalent pathogens in low birth weight infants e.g., Staphylococcus epidermidis which was the most infective agent in this study.

2. Impairment of other immune system components such as neutrophils, complement and fibronectin may limit the effect of the IVIG used.

3. Total serum IgG concentration and IgG subclass may not have been maintained at effective levels.

Further studies in neonates are necessary to determine the efficacy of IVIG in different clinical settings, the most effective dosing schedule, and the optimal level of organism-specific antibody. Standardisation or establishment of minimum levels of organism-specific activity per individual batch, though difficult, may be necessary to ensure the infusion of sufficient organism-specific antibody. Alternatively, specific hyperimmune or monoclonal antibody preparations may be required to provide reliable sources of effective treatment to the neonates with bacterial sepsis.

Antibody level against pathogens such as group B streptococcus, E. coli and S. epidermidis may vary from batch to batch of the same IVIG or between the different IVIG preparations available on the market. It would be useful in further studies to know the most likely organisms causing infection in the study population so that IVIG containing a specific antibody is used. This may be the reason why several multicentre trials using pooled IVIG have failed to show any statistical improvement in outcome when IVIG is given prophylactically. Further studies should use specific IVIG and should compare its use prophylactically and as therapy.

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