A LONGITUDINAL FOLLOW UP OF NATURAL IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* INFECTION IN CHILDREN LIVING IN AN ENDEMIC AREA OF WESTERN KENYA

By

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IS IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN IMMUNOLOGY
OF
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MAY 1997

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

To my dear husband and daughters Naomi and Stella for their understandig and patience. To my parents, brothers and sisters. You couldn't do better.

ACKNOWLEDGEMENTS

I would wish to give my very sincere thanks to all of you who in one way or another contributed to the success of this study. Sincere gratitude to Prof. A.S.S Orago, my university supervisor for his assistance and guidance. I am especially thankful to Dr. V. Udhayakumar for his guidance and support for this work. Gratitude also goes to Dr. Nahlen, the Director CDC/KEMRI, for allowing me to work in the CDC laboratories and the use of CDC facilities. I would also like to thank Dr. Altaf Lal and Dr. Bill Hawley of CDC for their support in carrying out this work. I appreciate all the financial assistance by CDC for my training. My sincere gratitude to my friends Caroline and Pauline who made my life in the laboratory very enjoyable. My gratitude also goes to all the laboratory staff; David Anyona, Linus Odawo, Zedekiah Nyawade, Thomas Otieno, Dickens Olang, Walter Aduda and Joseph Nyamweya for their great help and for making work in the laboratory easy and enjoyable. Thanks to all the Asembo Bay volunteers without whom the study would not have been possible. I am very greatfull to Kenyatta University for having awarded me a scholarship. Finally I would like to pass my sincere gratitude to all the members of my family for their patience and understanding.

TABLE OF CONTENTS

2.0 **OBJECTIVES OF THE STUDY**

3.0 **MATERIALS AND METHODS**

LIST OF FIGURES

ABSTRACT

The mechanisms of naturally acquired immunity to malaria are not clearly understood. In order to understand the developement of natural immune responses to malaria, I carried out a longitudinal follow up of young children less than 2 years of age in an area of western Kenya which is holoendemic for malaria. In this study, parasitaemia, body temperature, haemoglobin levels and cellular immune responses to Plasmodium falciparum exo-antigens and a recombinant antigen representing the 19 kD C-terminal domain of the merozoite surface protein (MSP-1 19 kDa antigen) of 49 infants were followed at monthly intervals.

The proliferative responses were measured by thymidine incorporation assay of peripheral blood mononuclear cells (PBMCs) isolated from finger prick blood. This response was measured longitudinally at 3-6 time points for each infant tested. Longitudinal analysis of proliferative responses to MSP-1 19kDa antigen indicated that most of the children 36/49(73.47%) responded at least once with a stimulation index value more than 2. Although many of these children had detectable levels of parasitaemia at several time points, they showed positive proliferative responses only a few times during the entire testing period. This finding suggests that cellular immune responses to MSP-1 19 kDa and exo-antigens are not long lasting. No correlation was observed between response to MSP-1 19kDa/exo-antigens and the prevailing infection status.

Levels of IL-4 (a TH2 cytokine) and IFN-γ (a TH1 cytokine) were measured in plasma samples and in culture supernatants collected from a subset of the study group. PBMCs from 79 infants were stimulated with MSP-1 19kDa antigen and exo antigens and the resulting supernatant tested for IL-4 and IFN-γ.

The results of this study show that a larger number of infants responded to MSP-1 19 kDa/exo-antigens by production of IL-4 than IFN-γ. Analysis of plasma samples also showed more individuals with detectable IL-4 than IFN-γ. There was however no correlation between either <u>in vitro</u> cytokine production or cytokine prevalence in plasma and the prevailing infection status.

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 THE MALARIA PARASITE:

Human malaria is caused by four parasite species of the genus Plasmodium namely P. vivax, P. ovale, P. falciparum, and P. malariae (Donno L., 1984). Plasmodium vivax causes most malaria infections in the world but P. falciparum causes the most severe disease and if left untreated, it has a high mortality rate in non-immune individuals (Phillips, 1983). Malaria is transmitted by female anopheline mosquitoes during a blood meal.

1.2 GLOBAL IMPACT OF MALARIA

Malaria continues to be one of the world's most widespread infectious diseases which kills 1 to 2 million children less than 5 years of age (Greenwood, 1990) and accounts for 300-500 million clinical cases annually (WHO, 1993). More than 90% of malaria-induced morbidity and mortality is caused by P. falciparum. In endemic areas, the most susceptible group involves pregnant women, children, and visitors from non-endemic areas (Marsh, 1992). Between 10-50 percent of people with celebral malaria die depending on the level of endemicity, level of care available and the age of the patient (Rey et al., 1966; Stance et al., 1982).

1.3 LIFE CYCLE OF HUMAN MALARIA PARASITE

The life cycle of the human malaria parasite is complex. It involves 2 phases in human host and three phases in the mosquito vector.

A female Anopheles mosquito ingests sexual stage parasites during a blood meal. Asexual parasites are digested together with red blood cells while gametocytes develop further to form male and female gametes in the mosquito midgut. Fertilization of the female gamete by the male gamete forms a zygote in the mosquito stomach. The zygote becomes a motile ookinete which then enters the stomach wall and develops into an oocyst. The oocyst increases in size and later on bursts to liberate thousands of motile sporozoites into the body cavity of the mosquito from where many sporozoites reach the salivary glands. The female mosquito now becomes infective. It pierces the human skin when feeding and injects sporozoites through the wound into the blood stream of the host.

In the human host sporozoites enter the parenchymal cells of the liver where they undergo pre-erythrocytic schizogony to form merozoites. After about 6-16 days post-infection, the envelope of the cell containing the schizont ruptures and merozoites are released into the blood circulation. The merozoites invade red blood cells within which they develop into ring forms. The rings develop to form trophozoites which then mature into schizonts

containing a number of merozoites. When schizogony is complete, the red cells rupture releasing merozoites into the blood stream. The merozoites then reinvade fresh erythrocytes in which another generation of parasites is produced by the same process. The schizogonic periodicity is 48 hours in ovale, vivax and falciparum malaria and 72 hours in quartan malaria.

Some merozoites give rise to sexually differentiated forms called gametocytes. Gametocytes invade fresh erythrocytes and they grow leaving the nucleus undivided (Bruce-Chwatt 1980; Louis et al.,1994).

1.4 CLINICAL PRESENTATION AND COMPLICATIONS IN MALARIA

The clinical features of malaria are exclusively associated with fever being the most common symptom of plasmodial species that infect man. Malarial fever represents a major cause of morbidity worldwide (Michael and Allan, 1994). It is caused by the release of cytokines like tumour necrosis factor alpha (TNF-α) that upregulate body temperature by stimulating prostaglandin E synthesis in the thermoregulatory center of the hypothalamus (Dinarello, 1987). The symptoms of malaria vary in relation to the species of the infecting parasite and in relation to the geographical settings (Miller et al., 1994). Falciparum malaria begins with headache, joint and back pains, prostration, a feeling of chill, nausea, vomiting or a mild diarrhoea, which increases in

intensity as the disease progresses. The spleen and the liver may become enlarged and there may be slight jaundice.

Cerebral malaria can be defined as altered consciousness in patients infected with P. falciparum (WHO, 1990). P. falciparum accounts for most of the severe manifestations of malaria and causes most of the mortality. Although in endemic areas cerebral malaria occurs only in children (Molyneux et al., 1991a); the peak incidence being between ages 3-4 in areas of low endemicity (Marsh, 1992), in non-endemic areas it can affect all age groups. The causes of cerebral malaria is unknown. Histological studies have shown that parasite sequestration on the cerebral vasculation could affect the blood flow causing cerebral malaria (MacPhersson et al., 1985). There is evidence that TNF-α may also contribute to cerebral malaria pathogenesis. Circulating TNF-α levels are elevated in cerebral malaria patients than in those with uncomplicated disease (Grau et al., 1989; Kwiatkowski et al., 1990). It has been proposed that TNF-α may contribute to cerebral malaria by enhancing cytoadherance of parasitized erythrocytes thus upregulating adhesion molecules like CD36, thrombospondin or intercellular adhesion molecule (ICAM-1) (Howard, 1992).

Malarial anaemia is multifactorial in origin. Destruction of erythrocytes during malaria infection could be a factor. Involvement of other factors has also been proposed. A decrease in bone marrow erythropoiesis during acute infection has also been

noticed (Abdalla et al., 1980; Phillips et al., 1986a). Experimental studies using P. vinkei model showed tha TNF-α was implicated in this bone marrow depression and erythropoiesis (Clark and Chaudhri 1988). These various mechanisms combined with parasitaemia (Abdalla et al., 1980) causes anaemia and death particularly in high endemic areas (Lackritz et al., 1992).

Non cerebral complications include hypoglycaemia. This may cause death or permanent brain damage (Kawo et al., 1990). It presents with confusion, coma and convulsions thus mimicking cerebral malaria. Other non-cerebral complications are pulmonary oedema and renal failure (WHO, 1990). Pulmonary oedema is associated with high mortality rate (WHO, 1990) and leads to increased pulmonary vascular permeability while renal failure has the pathological characteristics of acute tubular necrosis. Other complications are P. malariae glomerulopathy and hyperactive malarial splenomegaly (HMS).

1.5 IMMUNITY TO MALARIA

Immunity to malaria may be natural (innate) or acquired. Innate immunity does not require prior exposure to antigens in order to be effective. On the other hand, acquired immunity develops due to interactions with the antigens in question.

1.5.1 INNATE IMMUNITY

The best known examples of innate immunity to malaria are genetic disorders that are known to confer protection against disease or infection.

The relative resistance of West Africans and American blacks to infection with P. vivax is due to the absence of Duffy blood group determinants (Fya and Fyb) in these populations which are known to be receptors for the invasion of merozoites (Weatheral, 1987; Nagel, 1991).

Sickle cell trait is another genetic disoder known to provide resistance against severe malaria. This defect is lethal in its homozygous state (SS) as in sickle cell anaemia, though harmless in its heterozygous state. The heterozygous state causes sickling of erythrocytes at low oxygen tension, but heterozygotes are advantaged against severe P. falciparum malaria (Gendrel et al., 1992). However, sickle cell trait does not provide protection against infection.

Thalasaemias are the most common genetic disorders of the haemoglobin (HBE) in man; which are of different genotypes depending on the range of globin gene variants. There is evidence favouring selection by thalasaemias and hence protection against P. falciparum malaria (Hill, 1992; Luzzi et al., 1991)

In normal humans, foetal stage-specific gamma-globulin genes are silenced after birth and are not expressed in the adult. Exeptions are seen where fetal haemoglobin persists. This causes elevation of beta thalasaemia and sickle cell anaemia both of which confer protection against severe attacks of P. falciparum malaria (Berry et al., 1992)

Certain HLA class I and class II alleles have been found to protect against the development of severe malaria (Hill et al., 1991). Other protective genetic deformities are such as glucose-6-phosphate dehydrogenase defficiency and ovalocytosis.

Asexual blood stages of P. falciparum have been shown to be sensitive to nitrite (NO_2-) and nitrate (NO_3-) produced by macrophages (Stuer and Marleta, 1985) and neutrophils (McCall et al., 1989). The mechanism of the antiparasite effects of nitrogen oxide remains to be clearly established (Liew and Cox, 1992).

1.5.2 ACQUIRED IMMUNITY TO MALARIA

Acquired immunity to malaria is both species and stage specific (Howard, 1986). Thus, humans immunized with irradiated sporozoites of P. falciparum were refractory to challenge with sporozoites of this species, but not those of P. vivax. Further more these vaccinees were fully susceptible to challenge with asexual blood stage parasites of P. falciparum (Clyde et al., 1975)

Protection against malaria involves both cellular and humoral immune mechanisms. Humoral immunity is mediated by antibodies while cellular immunity is mediated by T cells. Both antibodies (Bouharoun-Tayoun et al., 1990; Newbold et al., 1992), effector T cells (Brake et al., 1988), and NK cells (Orago and Facer, 1991) play important roles. Relative roles of the different arms of the immune system vary greatly in different models, depending on parasite species, genetic constitution of the host and the hosts immune status (van der Weid and Langhorne, 1993).

1.5.2.1 Antibody Mediated Immunity to Malaria

That antibodies are important factors in anti-parasite immunity in P. falciparum malaria was shown by passive serum transfer studies in Gambian children who recieved immune IgG from adults living in malarious regions. These children were shown to be protected against P. falciparum infection (Cohen et al., 1961).

Several animal studies have shown protection by antibodies. Antibodies to the circumsporozoite protein have been shown to prevent liver cell infection (Zavala, 1991) thus protecting against sporozoite-induced malaria infection (Potocnjak et al., 1980; Egan et al., 1987). This type of immunity is species specific (Clyde et al., 1975). Antibodies against sexual stages are important for transmission blocking immunity by inactivating the parasite in the mosquito gut (Targett, 1990). This prevents infection of humans by infected mosquitos. In hyper- and holo-endemic areas, infants born to immune mothers are protected against malaria during the first six months of life, reflecting a transfer of protective antibodies from the mother (McGregor, 1984; Chizzolini et al., 1991).

The mechanism by which antibodies confer protectin is not certain. They may exert protection along different pathways; they may prevent parasite binding to host cell receptors for example sporozoite/liver cell interaction (Hollingdale et al., 1984) or invasion of erythrocytes by merozoites (Quinn and Wyler, 1979). Sporozoites that manage to invade liver cells in the presence of anti-sporozoite antibodies have been shown not to develope normally and they fail to release merozoites (Mazier et al., 1986).

Antibodies can mediate their effect of protection by being involed in antibody mediated cellular cytotoxicity (Lunel and Druihle, 1989;Bouharoun-Tayoun and Druihle, 1992). An example is when antibodies bind to antigens on infected erythrocytes enabling

recognition of parasites by neutrophils and macrophages via Fc receptors (Taliaferro and Cannon, 1936). Opsonizing sera was also found to be necessary for mouse peritoneal macrophages to ingest P. knowlesi-infected erythrocytes (Brown 1971). Phagocytosis of P. falciparum by macrophages has also been shown in vitro (Celada et al., 1982).

1.5.2.2 Cell-Mediated Immunity to Malaria

Cell-mediated immunity is primarily mediated by T lymphocytes either directly or indirectly through various effector mechanisms. T helper cells or CD4+ T cells play a central role in most immune reactions and are necessary for the induction of cytotoxic T cells and most B cell responses. Several studies have shown that CD4+ T cells with or without B cells play a crucial role in the development of immune protection of mice against blood stage infection (Troye-Blomberg et al., 1994). Cloned lines of CD4+ cells can adoptively confer protection and abrogation of immunity following CD4+ cell depletion from immune mice suggests that T cells alone may afford significant protection (Brake et al.,1988; Kumar et al., 1989). CD4+ T cells can be divided into TH1 and TH2 cells following antigenic stimulation, depending on the cytokines they produce (Mosmann and Coffman, 1987). There is evidence that suggests that CD4+ T cells of the TH1 phenotype are important during the acute phase of P. chabaudi infection (Langhorne et al., 1989). Control of this parasite during later phases also involves CD4+ cells of the TH2 type which provide help to B cells by

producing IL-4 and other lymphokines. In this model both cell-mediated immunity and humoral immunity acted sequentially to clear the infection (Langhorne et al., 1989; Stevenson and Tam, 1993). On the other hand, immunity to P. vinkei vinkei was found to be dependent on CD4+ T cells by production of IFNγ,which is a TH1 dependent process (Kumar et al., 1989).

The other type of T lymphocytes that mediate cellular immunity are the CD8+ Cytotoxic T cells which have been shown to play a major role in destroying liver cells infected with malaria parasites. Treatment of immunized mice with antibodies that destroy cytotoxic T cells renders them susceptible to sporozoite-induced malaria (Schofield et al., 1987b; Weiss et al., 1988). Administration of these cells can protect mice from sporozoite-induced malaria infection (Romero et al., 1989).

T lymphocytes recognise fragments of antigen presented as a complex with the major histocompatibility proteins on the surface.This way, T lymphocytes are able to inhibit growth of P. falciparum in vitro (Brown et al., 1986; Fell et al., 1994). However, while T cells can control parasitaemia, antibody may be required to eliminate parasites (Kumar et al., 1989; van der Weid and Langhorne 1993; Taylor-Robinson and Phillips 1994) but the process is under T cell control.

1.5.2.3 DICHOTOMY OF CYTOKINE PRODUCTION BY T-LYMPHOCYTES

CD4+ T cells or T helper cells play a central role in most immune reactions and are required for the induction of cytotoxic T cells as well as B cell responses. Exposure to certain antigens influences CD4+ T cell development with the result that they express characteristic phenotypes based on the cytokines they secrete (Mosmann and Coffman, 1987). Three distinctive phenotypes are known. These are THO, TH1 and TH2 phenotypes. TH0 cells are probably precursors and secrete IL2,IL3, IFNγ, IL4, IL5, IL6, and IL10. After antigenic stimulation, more defined subsets emerge; TH1 which secretes IL2 IFNγ and IL12 and TH2 which secretes IL4, IL5, IL6 and IL10 (Mosmann and Coffman, 1989). The existance of these two major functionally distinct TH cell subsets is also demonstrated in humans (Romagnani, 1991).

TH1 and TH2 cells cross regulate each others expressions. TH1 cells produce IFNγ which preferentially inhibits proliferation of TH2 lymphocytes (Gajewski et al., 1989). On the other hand, both IL4 (Swain et al, 1990; Seder et al., 1992) and IL10 inhibited TH1 development. IL10 down-regulates cytokine synthesis in TH1 lymphocytes (Mosmann and Moore, 1991). This results in the predominance of either TH1 or TH2 cells. In addition cytokines produced by CD4+ T cell subsets can inhibit the effector activities induced by the opposing subset. TH2-produced cytokines IL3, IL4, and IL10 inhibit IFNγ dependent macrophage activation and

extravascular killing of parasites such as Leishmania major and Toxoplasma gondi (Gazinelli et al., 1989)

TH1 responses are characterised by a strong cell-mediated immune response and often only a low humoral response while TH2 responses are characterised by high titre antibody responses but poor delayed type hypersensitivity reactions (Mosmann and Coffman 1989). In addition, TH1 cells in humans were found to be cytolytic (Parronchi et al., 1992)

It has been shown that cytokines released by T cells play a role in isotype switching, for instance, IL4 which is a TH2 cytokine, allows B cells to switch from IgM to IgG secretions (Snapper and Paul, 1987). Further IL4 results in switching from IgG1 to IgE in mice (Snapper and Paul, 1987) and in humans (Mills et al., 1992). IFNγ on the other hand is associated with switching from IgM to IgG2a.

Development of an appropriate TH subset during an immune response is important because certain pathogens are effectively controlled by either a predominant TH1 or TH2 immune response (Sher and Coffman, 1992). The development of the TH subset may involve innate immune cells like macrophages, natural killer cells, or mast cells since the host interacts with the pathogen bofore the development of specific immunity (Kullberg et al., 1992). It has also been suggested that the switch from TH1 to TH2 may be due to

a switch in antigen presentig cells (from macrophages to B cells) (van der Weid and Langhorne 1993; Taylor-Robinson and Phillips 1994).

In the malaria situation, the balance between T cell subsets producing different cytokines upon activation is important for the development of immunity to the blood stages of malaria parasite (Perlmann et al., 1995) Earlier work using the P. chabaudi model indicated that during the primary parasitaemia of P. chabaudi infection, TH1-type response predominated, followed by a TH2-type response during the late phases of infection (Langhorne et al., 1989).

1.5.2.4 ROLE OF CYTOKINES IN INFECTIOUS DISEASES

The importance of the TH subset in determining the outcome of an infection has been shown in certain parasitic diseases such as Leishmania major infection in mice. Resistance to this infection is characterised by a strong delayed-type hypersensitivity reaction and low antibody titres. This implies that TH1 responses are protective while TH2 exercebate the disease (Heinzel et al., 1991; Salgame et al., 1991)

In human leprosy, T cells from slowly progressing tuberculoid lesions were found to be of the TH1 type while those from fatal lepromatous leprosy were of TH2 type (Salgame et al., 1991).

Nematodes like Nipostrongylus brasiliensis or schistosomes in mice elicite a TH2 response chracterised by the expansion of TH2 cells, high IL4 levels and switching to IgE (Sher and Coffman, 1992; Urban et al., 1992). Abrogation of this response however, did not affect protective immunity to these infections implying that TH2 cell activation did not provide protection (Sher and Coffman, 1992; Urban et al., 1992).

Peripheral blood lymphocytes (PBL) from persons chronically infected with Toxocara canis or those allergic to certain allergens generated mainly TH2 clones after antigen specific stimulation in vitro. Lymphocytes from patients with tuberculosis gave rise to TH1 clones in vitro. The protective roles of these T cell clones is however not clear.

Treatment with anti-IL4 decreases resistance to Heligomosomoides polygrus, suggesting that IL-4 release by TH2 cells is involved in protection (Urban et al., 1992).

These data suggest that TH cells can be directed towards developing into TH1 or TH2 cells dependig on the nature of the immunogen and the genetic background of the host. TH1 cells are often protective especially in cases of intracellular infections. The role of TH2 on the other hand is unclear.

1.5.2.5 CYTOKINES IN MALARIA

The question of TH1 and TH2 in malaria immunity has been investigated in rodent models. This work indicated that during primary parasitaemia of P. chabaudi infection in mice TH1 cells predominate followed by a TH2 response during the late phases of the infection (Langhorne et al., 1989, 1990)

A range of different cytokines are produced during the course of rodent (Clark et al., 1981;Bate et al., 1989) and human malaria (Grau et al., 1989; Kwiatkowski et al., 1989). Interactions between cytokines such as IFN-γ Tumour necrosis factor-alpha (TNF-α) and many effector cells are considered as important for the outcome of infection with malaria (Orago and Facer, 1991). These cytokines may have a direct cytotoxic effect on P. falciparum growth and development (Orago and Facer, 1993).

IFN-γ is a product of TH1 cells and has been shown to be crucial for killing of intra-hepatic and intra-erythrocytic parasites probably by inducing monocytes and macrophages to release nitric oxide (Hoffman et al., 1989). IFN-γ levels were shown to be high in children with malaria but this was not associated with disease severity (Kwitkowski et al., 1990) but may have a key role in immune protection against P. falciparum infection. This is supported by epidemiological studies in Madagascar where protected individuals had higher serum IFN-γ levels than non-protected individuals (Deloron et al., 1991). On the contrary, work by other

scientists suggested that IFN-γ production by T cells may not be effective at controlling infection. In some cases, it may actually contribute to disease (Riley et al., 1991).

Malaria parasite antigens stimulate monocytes and macrophages to release TNF-α (Dinarello et al., 1986; Kwiatkowski et al., 1989) in bursts that coincide with schizont rupture (Scuderi et al., 1986; Kwiatkowski et al., 1989) in patients with malaria fever. This is evidence that TNF-α is a mediator of the fever that is associated with schizont rupture (Kitchen, 1949). In addition to this, high TNF-α levels have been found to be a better predictor of fatal cerebral malaria and is also associated with hypoglycaemia in childhood cerebral malaria (Grau et al., 1989; Kwiatkowski et al., 1990). TNF-α stimulates a variety of cellular mechanisms that prevent parasite growth such as the release of free oxygen radicals by macrophages and neutrophils (Clark and Cowden, 1990). It may also act in concert with soluble factors present in serum of malaria fever patients (Mendis et al., 1990).

The other cytokine associated with malaria is IL-4 which enhances production of reactive oxygen intermediates by bone marrow macrophages. Other cytokines like IL1 and IL-6 derived from macrophages are also associated with severe malaria (Kwiatkowski et al., 1990; Molyneux et al., 1991a).

The association between pathology in human malaria and cytokine

expression is convincing (Kwiatkowski et al., 1990) and thus in summary, there is reason to believe that T cells can eliminate parasites, the consequence of which is activation of these T cells and liberation of cytokines that result in disease (Good, 1995).

1.6 ATTEMPTS TO COMBAT THE DISEASE.

Currently, prevention of disease is dependent on avoiding contact with mosquitoes or on chemoprophylaxis and the use of insecticide. Antimalarial drugs are used to prevent the onset of disease, treat clinical cases and to prevent disease transmission. Use of drugs such as quinine, mefloquine and others is widespread (Oaks et al., 1991). However, chemoprophylaxis has become less effecitve due to emergence of drug-resistant parasites. Chloroquine resistance has been observed in P. falciparum and P. vivax infections and there is also increasing resistance to other antimalarial drugs worldwide (Luxermburger et al., 1994). Other factors comprising drug use are such as toxicity and side effects apart from emergence of resistance (Carlson, 1993).

Onother important method of controling disease is the elimination of larval development caused by human activities such as irrigation, public works and construction projects. This may be done by draining and filling in bodies of water, use of petroleum and larvicides, and biological methods such as the use of larvivorous fish and toxin-producing bacterium called Bacillus-

thuringiensis israeliensis.

Residual insecticides such as DDT (Wyler D.J.,1993) applied on walls of houses are effective but they do not affect the mosquitoes that rest outdoors; and mosquitoes have become resistant to some pesticides and some have learnt to avoid insecticide-treated surfaces rendering the use of chemicals unsuccessfull (WHO, 1994b).

Protective clothing, insect repellents, mosquito coils and bed nets can help reduce human-mosquito contact.

Consequently, the main question in malaria research is whether it would be possible to develope vaccines to lessen disease (Louis et al., 1994). A promissing new measure is the use of vaccines against the sexual blood stages of the parasite (Valero et al., 1993). Vaccine development has however been made difficult by our little understanding of the nature of protective immunity, complex life cycle of the malaria parasite, antigenic polymorphism, antigenic variation and poor imunological responses to critical antigens.

1.7 RATIONALE FOR THE STUDY

In hyper- and holoendemic areas including parts of Kenya, infants born to immune mothers are protected against malaria due to transplacental transfer of protective antibodies for the first six months of life (Chizzolini et al., 1991). During the rest of the first five years of life, severe and often fatal infections are frequent. During this period, children in hyper-endemic areas are susceptible to severe life threatening complications of malaria. As age increases, the burden of disease diminishes. Although several immunoepidemeological studies have been conducted the natural mechanisms of protective immunity to malaria still remain unclear.

Several studies have shown that CD4+ (T helper) cells are crucial for the development of protective immunity to blood stage infection (Troye-Blomberg et al., 1994). TH1/TH2 balance has already been studied in rodent malaria using the P. chabaudi model (Langhorne et al., 1989; Langhorne et al., 1990) and the P. vinkei model (Kumar et al., 1989). The precence of TH1/TH2 subsets has also been shown in humans (Romagnani, 1991) and a range of different cytokines have been shown to be produced by these T cell subsets during the course of rodent (Clark et al., 1981; Bate et al., 1989) and human malaria (Grau et al., 1989; Kwiatkowski et al., 1989). It is not clear however whether there is a TH1/TH2 subset profile in human malaria, and if there is , whether it is protective. Characterisation of

protective CD4+ cells responsive to parasite antigen may facilitate anti-malarial vaccine development as it will provide a means of selecting antigens which induce approprite effector functions of the CD4+ lymphocytes.

In this study, the ability of MSP-1 antigen in inducing cell mediated immune responses and cytokine production was tested.

CHAPTER TWO

2.0 OBJECTIVES OF THIS STUDY

2.1 General objectives.

To carry out a longitudinal follw up of natural cellular immune responses in young children living in a malaria holoendemic region of western kenya, before, during and after acute episodes of P. falciparum malaria.

2.2 Specific objectives:

2.2.1. To develop practical methods of determining the cellular immune responses to malaria antigens in infants using finger prick blood.

2.2.2. To characterise the development of longitudinal lymphoproliferative responses to MSP-1 19kDa antigen and exo-antigens in infants.

2.2.3. To find out whether there is a TH1/TH2 polarization of cytokine responses to malaria antigen in young children.

2.2.4. To determine the plasma cytokine profiles for IL-4 and IFNγ in children during natural exposure to malaria.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY SITE

The study was conducted in an area of western Kenya called Asembo Bay which is holoendemic for malaria. Malaria transmission occurs all the year round, with peaks during the rainy seasons; short rains during the months of October and November and long rains during the months of March, April and May. The map of the study area is shown in Figure 1.

3.2 STUDY SUBJECTS

3.2.1 **Study population**

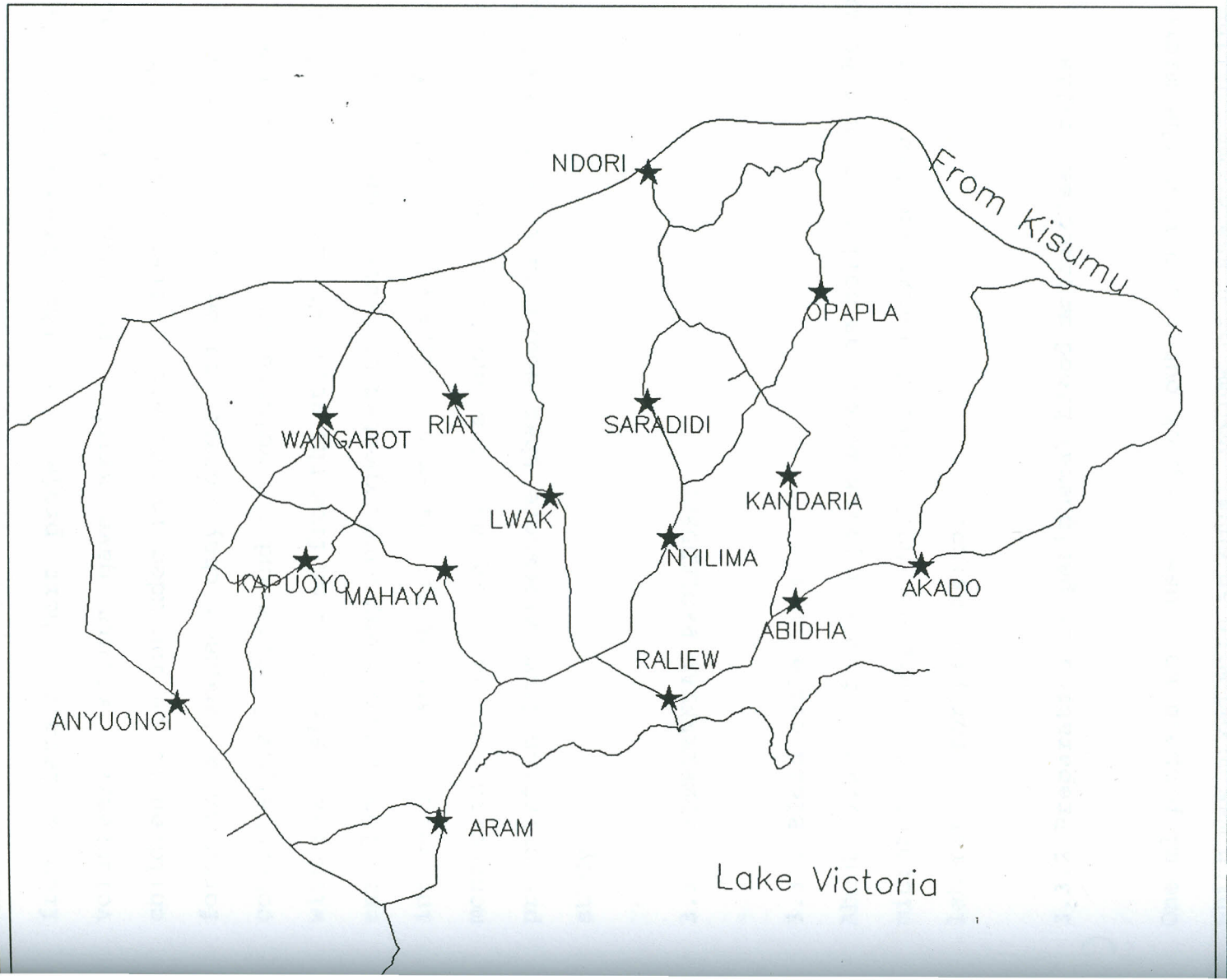
The study population was made up of 60 infants selected from the Asembo Bay Cohort Project, both males and females aged between 6 months and 2 years. Approximately 0.5ml of finger prick blood was collected from these infants once a month, or during clinical episodes of malaria. In this manner, children were followed for 6 months during their first 2 years of life.

3.2.2 **Exclusion and inclusion criteria**

The volunteers were infants born in and who were permanent residents of the Asembo Bay. They were both male and female between the ages of 6 months to two years and were picked at random

Fig 1:A MAP OF ASEMBO BAY

SHOWING STUDY VILLAGES



BNROADSS

ROADS

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from a larger cohort project at the Asembo Bay. All the volunteers' mothers gave written informed consent for their children to be included in the study after reading the consent forms in a language they understood best. The recruitment was completely voluntary and the volunteers' mothers had the right to withdraw their children from the study whenever they wished. Those enrolled in the study were expected to resieve general health care, including treatment for malaria. This was to allow for complete monitoring of the study subjects and to prevent them from taking prescription from elsewhere that would have interfered with the study.

3.3 EXPERIMENTAL PROCEDURES

3.3.1 Blood collection

About 0.5 ml of finger prick blood was collected into heparinized microtainers (Sarstedt Numbrecht, Germany) and transported to the laboratory for processing.

3.3.2 Preparation of peripheral blood mononuclear cells

One ml pippets were used to pick out blood from the microtainers which was mixed with an equal volume of 0.85% normal saline [Cross Laboratory Supplies, Kisumu, Kenya] then layered over 2mls of ficoll-hypaque [Pharmaclia Uppsala, Sweden] in 15mls disposable centrifuge tubes [Beckton Dickinson and Co., U.S.A.]. The blood

was then spun down in a GS-6R centrifuge [Beckman Instruments Inc. U.S.A.] at 1500g for 30 minutes and 25°C. Peripheral blood mononuclear cells (PBMCs) were recovered from the interphase of lymphoprep density gradient to give a lymphocyte-enriched cell suspension. The cell suspension was transfered into clean 15ml centrifuge tubes and washed twice with normal saline. After the final wash, the cells were resuspended in 1ml complete tissue culture medium made up of 10% human AB+ serum [CDC, Atlanta], 1% l-glutamine-200 mM [Life Technologies Inc. Grand Island, N.Y., U.S.A.] and 1% penicillin Streptomycin [Life Techinologies Inc. Grand Island, N.Y. U.S.A.] all in RPMI-1640 medium [Gibco BRL Life Techinologies U.K)

3.3.3 Total Leucocyte Count

The viability of the PBMCs preparation was determined by the trypan blue dye exlusion test. The cell concentration was determined using the following formula:

(No. of cells in one box) x (dilution) x (volume)

after which it was reconstituted to 1 million or 0.5 million cells per ml depending on the counts obtained.

3.3.4. Peripheral blood mononuclear cell (PBMC) cultures

Peripheral blood mononuclear cells (1×10^5 or 5×10^4) were cultured in a total volume of 0.2ml of complete RPMI in round-bottomed 96-well tissue culture plates [Costar Corporation, Cambridge, U.S.A.]. Three cultures were set up in triplicates; one with 1 microgram/ml E-KNG, another with 100 microlitres of parasite culture supernatant and another with plain or incomplete medium (control). Phytohemaglutinin (M form) [Life technologies Inc. Tuberculin Purified Protein Derivative S.U.S Units], [Connaught laboratories limited Ontario, Connade], [Statens serum institute, Artillerives 5-DK-2300, Copenhagen S. Denmark], was used as a positive control at a concentration of 10ug per ml . Cultures were maintained at 37°Cin an atmosphere of 5% CO_2 in a water jacketed incubator [NuAire, Inc. Minnesota, U.S.A].

3.3.5 Lymphocyte proliferation assays

After 6 days of culture , 150ul of culture supernatant from each well was collected and stored in nunc vials [Inter Med., Denmark] at -20°C for cytokine ELISA assays. This was replaced by an equal volume of complete RPMI-1640 medium. The cultures were then labeled with 1 uCi methyl-Thymidine [Amersham International plc. Amersham laboratories] and 8 hours later, the cells were harvested onto filter mats [Wallac OY., Turku, Finland], by means of a skatron cell harvester [Skatron, Norway]. The thymidine uptake

was assesed by a liquid scintillation counter [LKB Wallac, Turku, Finland]. The results were expressed as the stimulation index defined as mean counts per minute in the test cultures/ counts per minute in antigen free control cultures.

3.3.6 Criteria for symptomatic versus asymptomatic grouping of study subjects

The criteria for the division of the study group into symptomatic and asymptomatic was based on the temperature and the parasite density. The symptomatics were those who had a temperature greater than or equal to 37.5^{0}C and a parasite density greater than or equal to 2500 parasites per microlitre of blood.On the other hand, asymptomatics were those that had a parasitemia but with no fever. The non parasitemic group had no parasites at all.

3.3.7. Cytokine assays

The required number of ELISA plates [Immunol dynatech laboratories, Mcclean VA] were coated with 50 microlitres of anti-human IL4 polyclonal antibody [R and D systems, Minnesota, U.S.A.] or anti-human IFNγ neutralizing antibody (goat IgG) [R and D systems, Minnesota, U.S.A.] at a concentration of 5 micrograms per ml for both. They were then incubated at room temperature for 4 hours and then washed 4 times in PBS/Tween, with one minute incubation periode between the washes. The plates were then blocked with 100

microlitres per well of PBS/10% FBS for two hours at room temperature. After washing 4 times in PBS/Tween, cytokine standards and supernatant or plasma samples were added, 50 microlitres per well and then incubated overnight at 4°C. The plates were washed 4 times after which 100 microlitres of secondary monoclonal mouse anti-human IL4 (5 micrograms per ml) [R and D systems,Minnesota, U.S.A] or monoclonal mouse anti-human IFNγ (2ug/ml) [Genzyme, Cambridge MA, U.S.A] were added and incubated for 45 minutes at room temperature. After 6 washes in 200ul per well of PBS/tween, 100ul per well of a 1:500 dilution of the conjugate antibody, goat anti-mouse IgG-HRP [Southern Biotechnology Associates, Inc Birmingham AL. U.S.A] was added followed by a further 30 minute incubation periode at room temperature. The plates were washed 8 times after which 100ul of TMB substrate solution [Kirkegoard and Perry laboratories , Gaithesburg, Mayrland] was added. The colour reaction developed in about 15 minutes after which the reaction was stopped using sulphuric acid. The plates were read at 450 nanometres.

CHAPTER FOUR

RESULTS

4.1 PREPARATION OF PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) FROM FINGER PRICK BLOOD

The mean number of PBMCs obtained from 49 infants(observed at 1-3 time points each to a total of 120 time points) was 3.10±0.76 million cells per 1 ml of finger prick blood (range 2.34-3.86). The lowest count obtained was 0.64 million cells and the highest count obtained was 10.88 million cells per ml of blood.

4.2 LYMPHOPROLIFERATIVE RESPONSES TO PHYTOHEMAGLUTININ (PHA) MITOGEN IN INFANTS:

In the in vitro cellular immune responses, PHA, a mitogen known to activate human T cells was used as a positive control.Forty nine infants were followed (3-7 time points each) to a total of 213 cumulative incidences. In this study 96.71%(206/213) showed positive responses with a stimulation index (S.I.) greater than 2. The mean stimulation index (S.I.) was 27.81±7.59 (range 20.22-35.40) The lowest PHA S.I. was 0.1 (study number 3369) and the highest PHA S.I. was 243.33 (study number 2819). This result shows that PBMCs obtained from finger prick blood can be reliably used to measure cellular immune responses. Responses of PBMCs to PHA were compared to the presence or absence of parasites. It was found that the magnitude of response to PHA was not significantly altered by the parasite density ($r=0.0288$, $F=0.1755$, $n=213$; $P>0.5$).

Summary: Magnitude of response to PHA was not influenced by host malaria-infection status.

4.3 LYMPHOPROLIFERATIVE RESPONSES TO MSP-1 19kDa ANTIGEN

Earlier studies have shown that MSP-1 19 kDa antigen is an important target for malaria vaccine developement. In a recent study, antibody levels to MSP-1 19kDa antigen were found to correlate with clinical protection against P. falciparum infection (Oaks et al., 1991). In this study, the cellular immune responses to MSP-1 19kDa antigen was evaluated in infants who were naturally exposed to infection. A summary of the responses to MSP-1 19kDa antigen is given on Table 2. Forty nine infants were tested (3-7 time points each to a total of 287 cumulative incidences). From figure 2 it is evident that positive proliferative response to MSP-1 19kDa antigen (S.I.>2) constituted 20.69%(6/29) for the symptomatic, 14%(21/150) for the asymptomatic and 13.89%(15/108) for the aparasitaemic groups.There was also no difference in the mean S.I. between the groups: 1.42 (n=32) for the symptomatic, 1.18 (n=162) for the asymptomatic and 1.19 (n=113) for the non-parasitemic groups. When the parasitaemic and the aparasitaemic groups were compared, there was no difference in percentage positive responses (S.I.>2) between the groups;15.08%(27/179) for the parasitaemic group and 13.89%(15/108) for aparasitaemic groups (figures 3 and 4). Responses to MSP-1 19kDa antigen were compared to their respective parasite densities to determine any relationships between the two variables. No significant linear correlation was found ($r=-0.03$, $n=281$, $F=0.259$; $P>0.5$).

Fig 2
Lymphoproliferative responses to MSP-1 19 kDa antigen in young children in this study

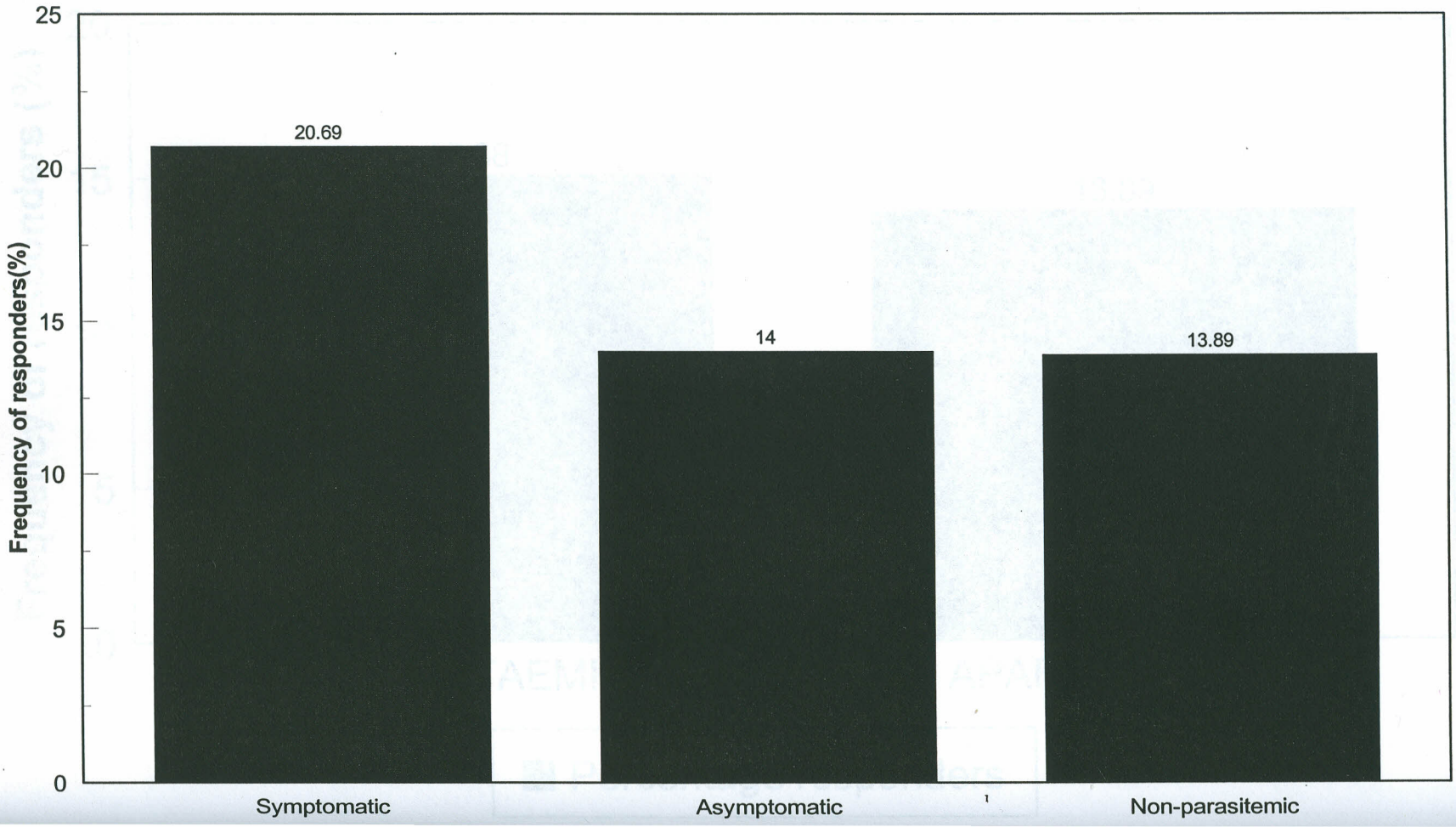


Figure 3
Positive proliferative responses to MSP-1 19KDa in parasitaemic and aparasitaemic infants in this study

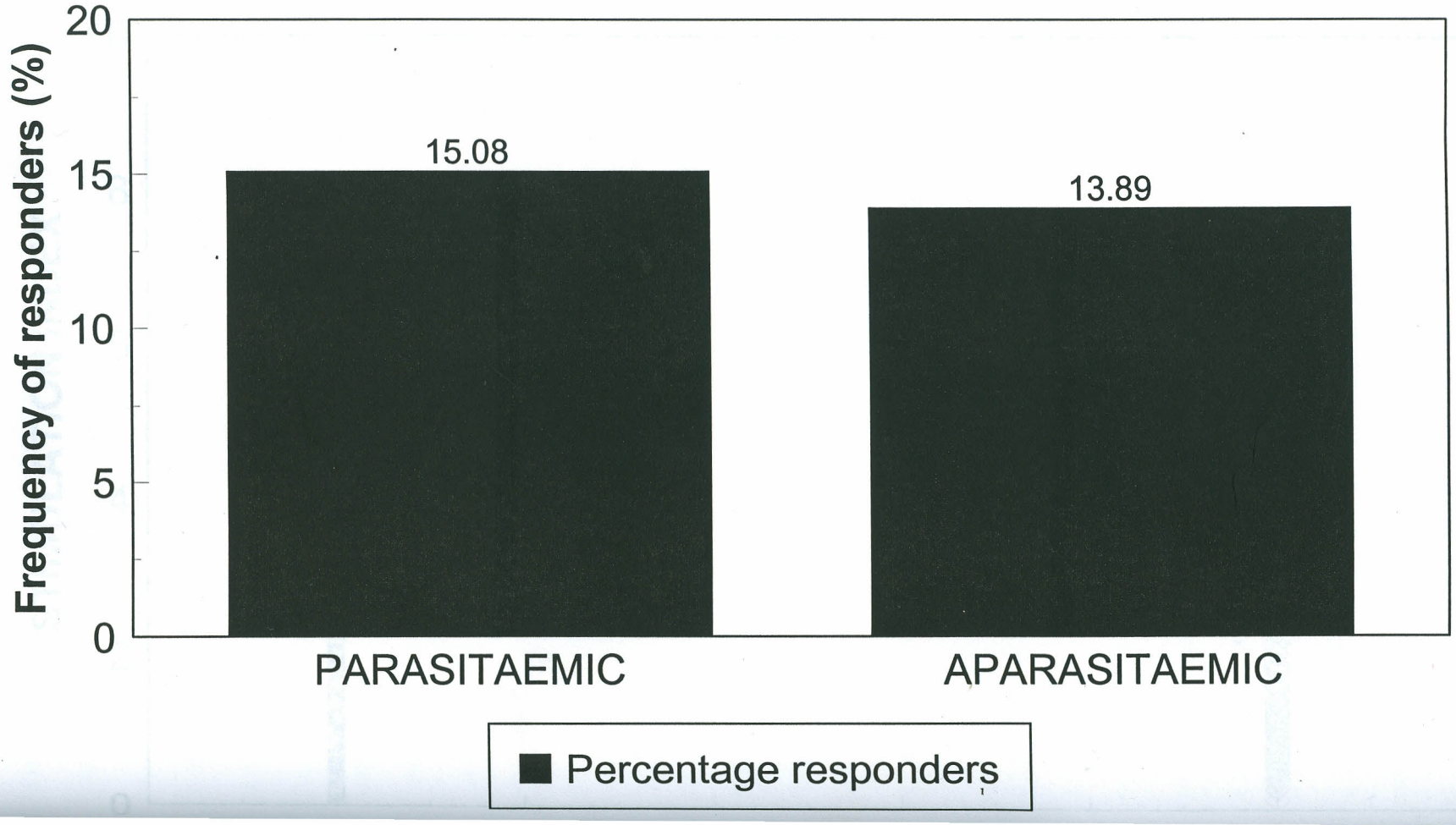
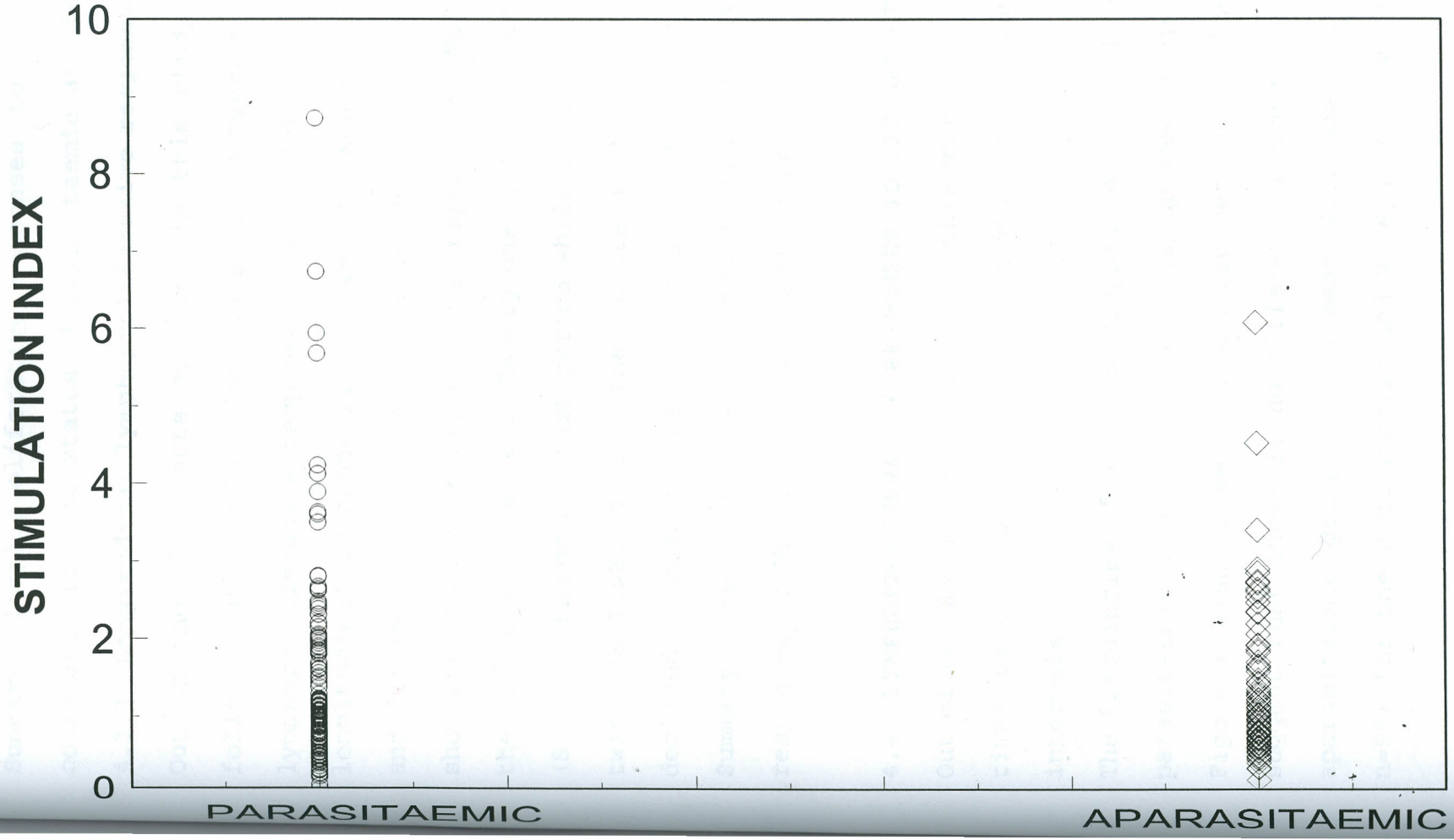


Figure 4
Lymphoproliferative responses to MSP-1 19KDa in relation to infection in infants in this study



Summary: Lymphoproliferative responses to MSP-1 19KDa did not correlate with the status of parasitaemia at the time of testing.

4.3.1 Longitudinal lymphoproliferative responses to MSP-1 19 kDa

Out of the 60 infants enrolled in this study, 37 infants were followed at monthly intervals to determine the longitudinal lymphoproliferative response to MSP-1 19kDa. Figure 5 shows the longitudinal lymphoproliferative resposes of 19 of these infants and figure 6 shows those of 18 infants. Sixteen of these did not show any positive proliferative responses to MSP-1 19kDa at any of the time points tested. Twenty one of them responded at least once (S.I.>2) during the test period while 6 of them responded at least twice (S.I.>2) during the entire test period. This response declined in most of the individuals and was not consistent.

Summary: The above results suggest that the lymphoproliferative responses to MSP-1 19kDa is short lived.

4.4 LYMPHOPROLIFERATIVE RESPONSES TO EXO ANTIGEN

Out of the 60 infants enrolled in this study, 49 were followed (3-5 times each to a total of 173 cumulative incidences) at monthly intervals.

The frequencies of positive responders (S.I.>2) were 29.59% for the parasitaemic and 26.67% for the aparasitaemic groups (Fig 7). Figure 8 also presents a similar set of results. These results suggest that there is no difference between the parasitaemic and aparasitaemic groups. The mean S.I. was 4.57±1.90 (2.67-6.47, n=99) for the parasitaemic and 2.26±0.80 (1.46-3.06, n=75) for

Figure 5
Longitudinal proliferative responses to MSP-1 19KDa in children between september 1995 to march 1996.

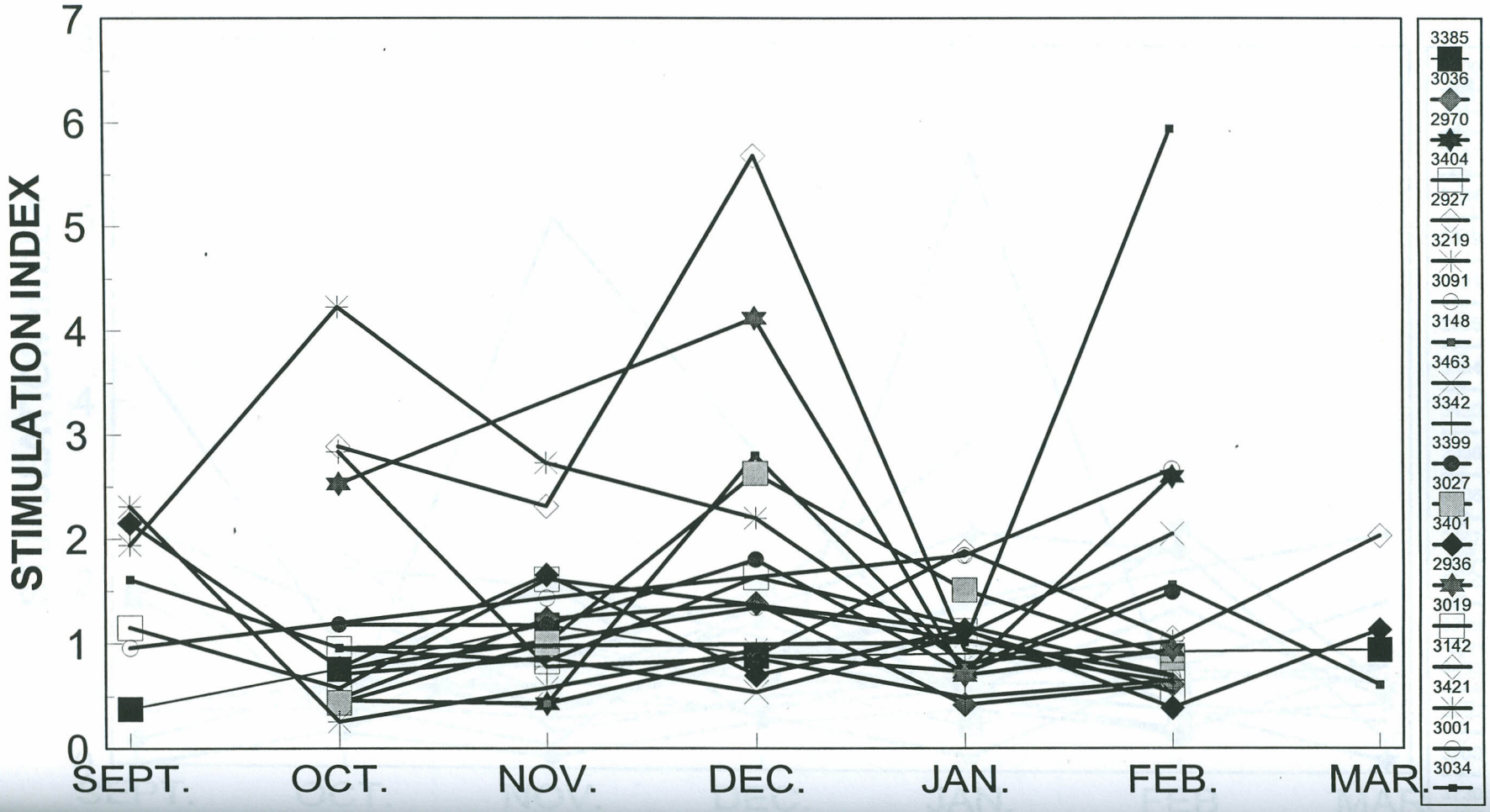


Figure 6
Longitudinal proliferative responses to MSP-1 19KDa in young children between september 1995 to march 1996

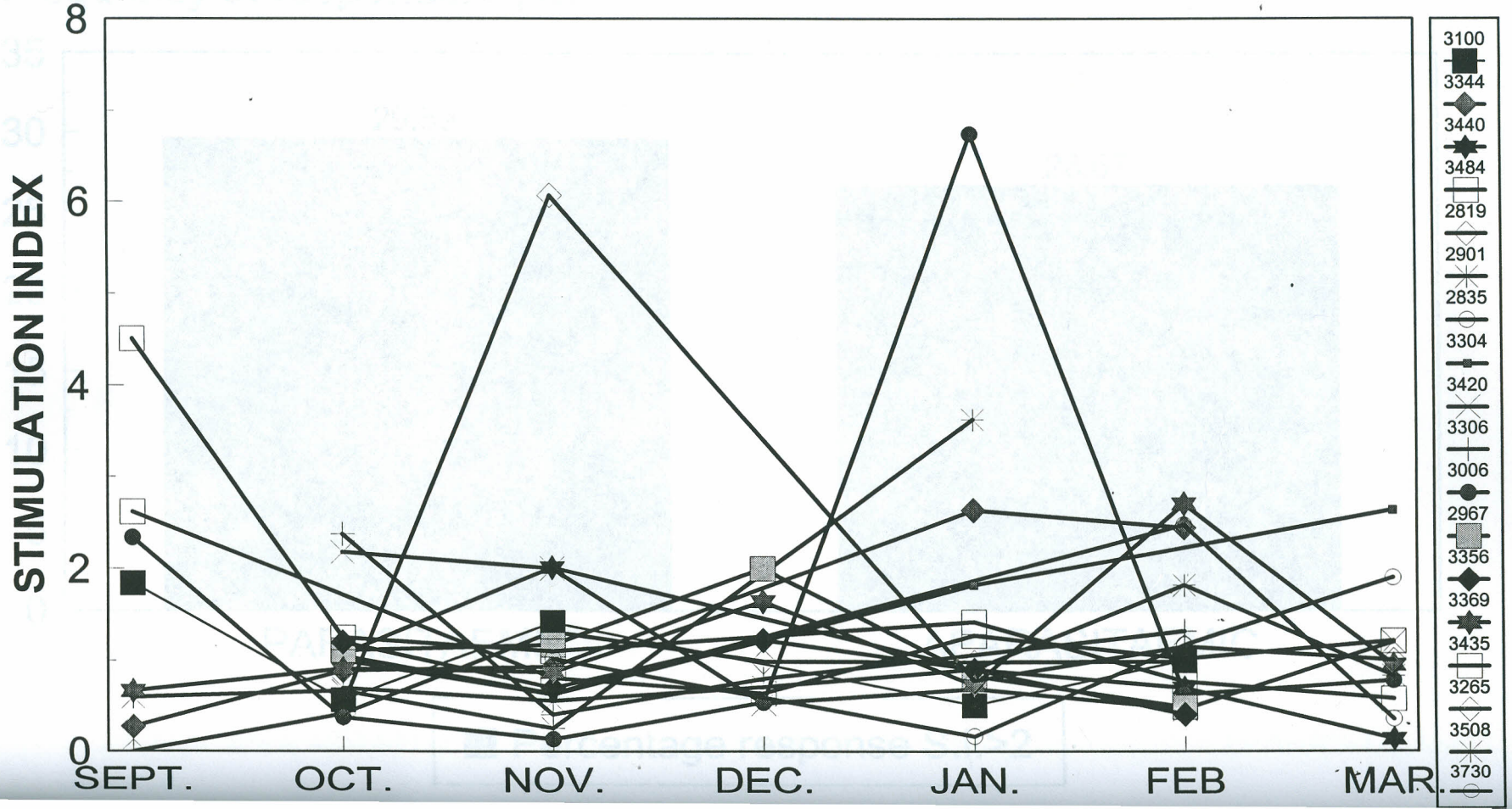


Figure 7
Lymphoproliferative responses to exo antigens in relation to infection in infants in this study

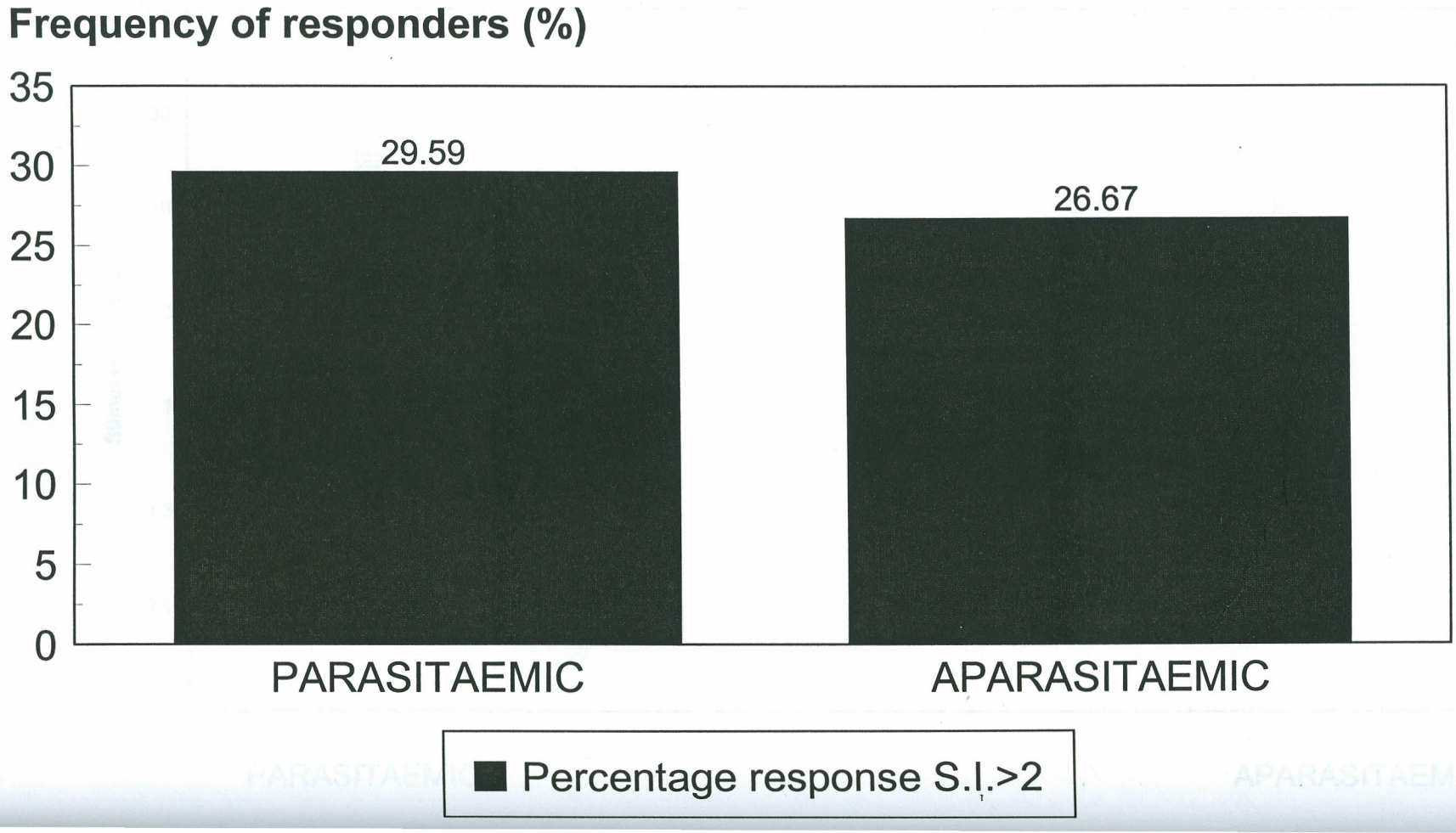
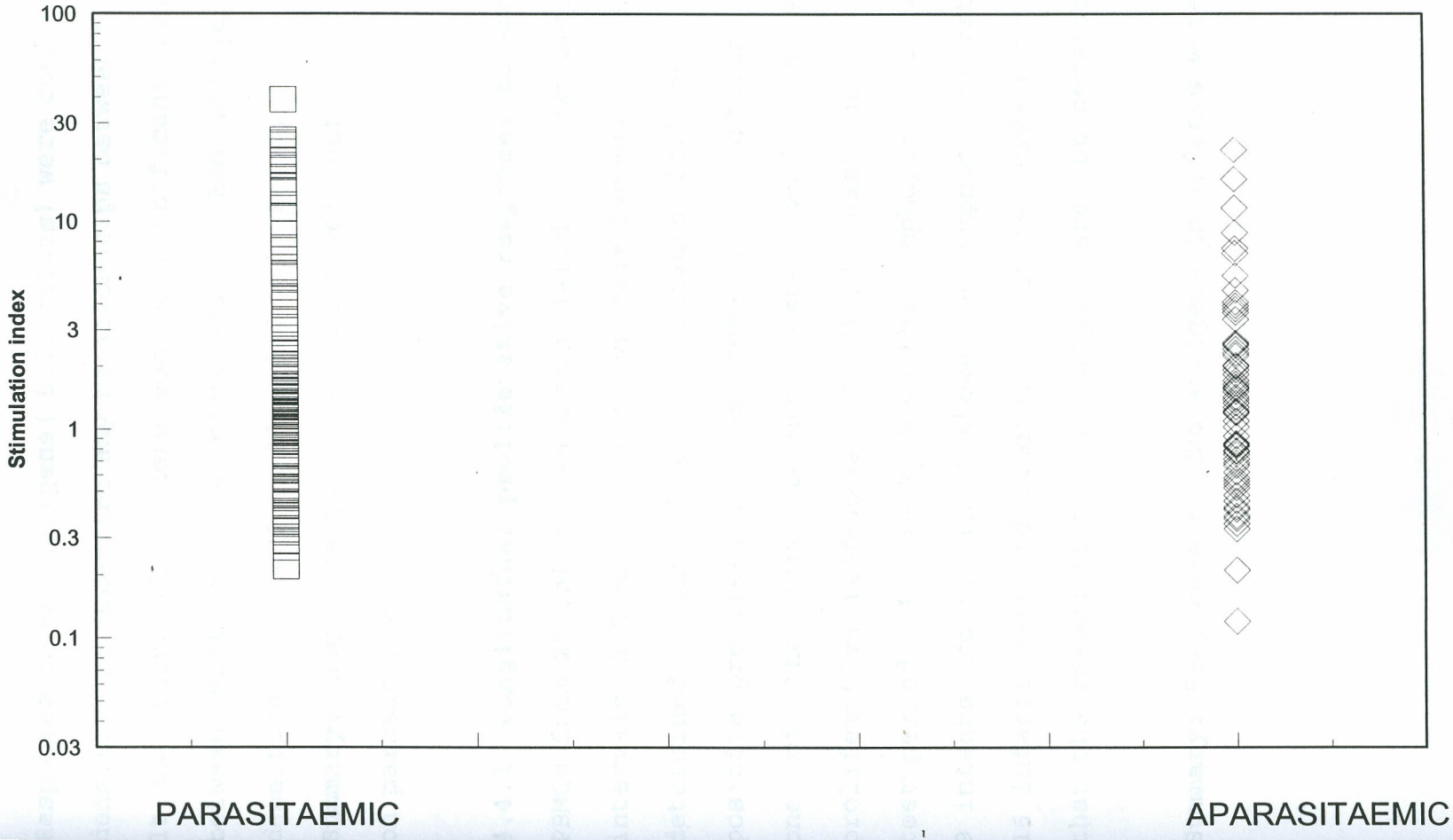


FIGURE 8
Lymphoproliferative responses to exo antigens in parasitemic and aparasitemic children in this study



the aparasitaemic groups.

Responses to exo antigens(S.I. values) were compared with parasite densities to determine any relationships between the two variables. It was found that there was no significant linear correlation between proliferative responses to Exo antigens and parasite densities ($r=-0.096$, $n=110$, $F=0.998$; $P<0.5$).

Summary: Responses to exo antigens were not found to be dependent on parasitemia.

4.4.1 Longitudinal proliferative responses to exo antigens

PBMCs from 25 infants were stimulated with exo antigens at monthly intervals (3-5 months each) and their lymphoproliferative responses determined. Out of the 25 infants followed, 7 did not show positive proliferative responses to exo antigens (S.I.>2) at any one of the time points tested while 18 showed positive proliferative responses(S.I.>2) at least once during the entire test period. Figure 9 shows the lymphoproliferative responses of 9 infants and figure 10 shows the lymphoproliferative resposes of 16 infants selected randomly. It is evident from these figures that the responses to exo antigens are not persistent.

Summary: Responses to Exo antigens in infants were short lived.

Fig 9
Longitudinal lymphoproliferative responses to exo antigens in children during this study

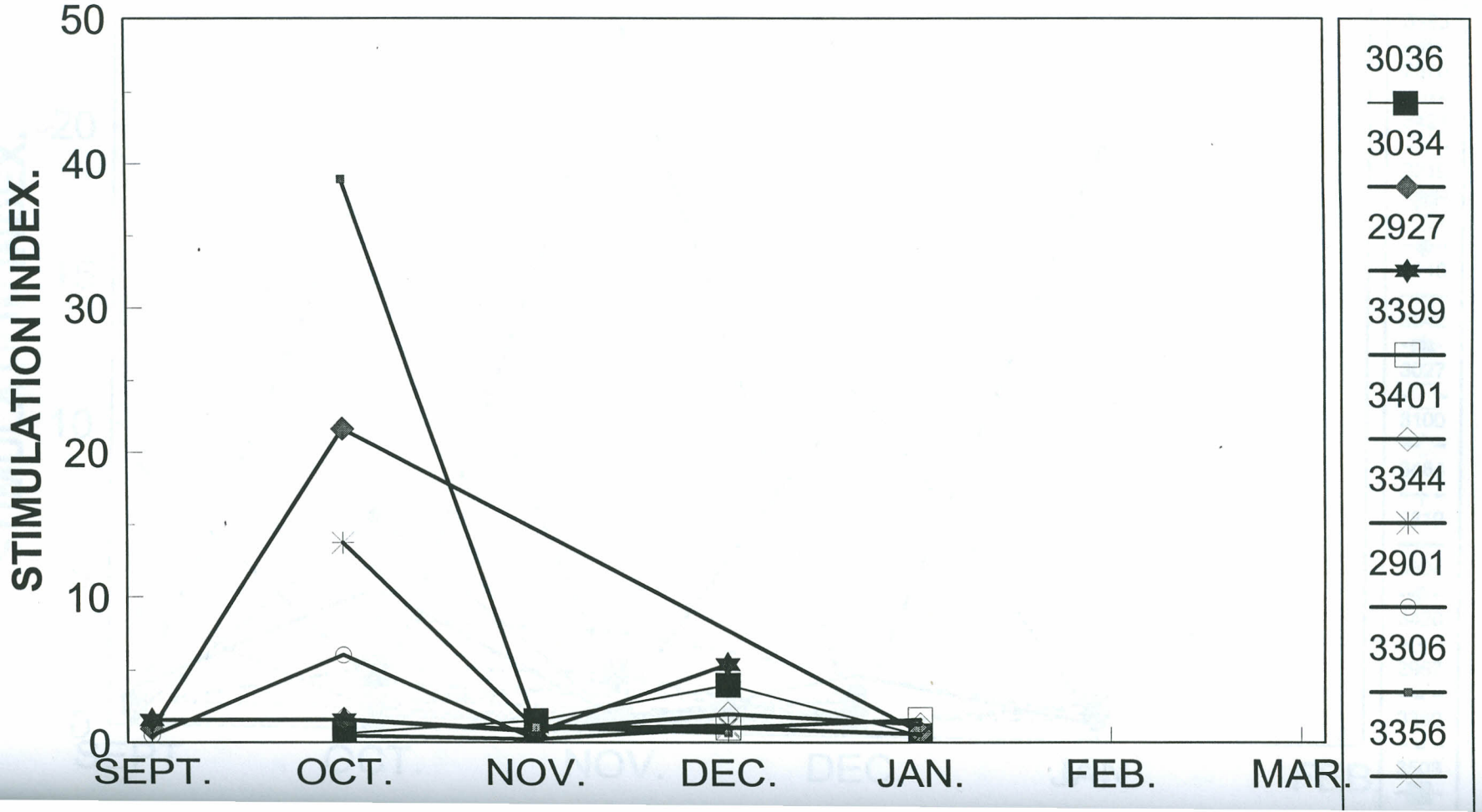
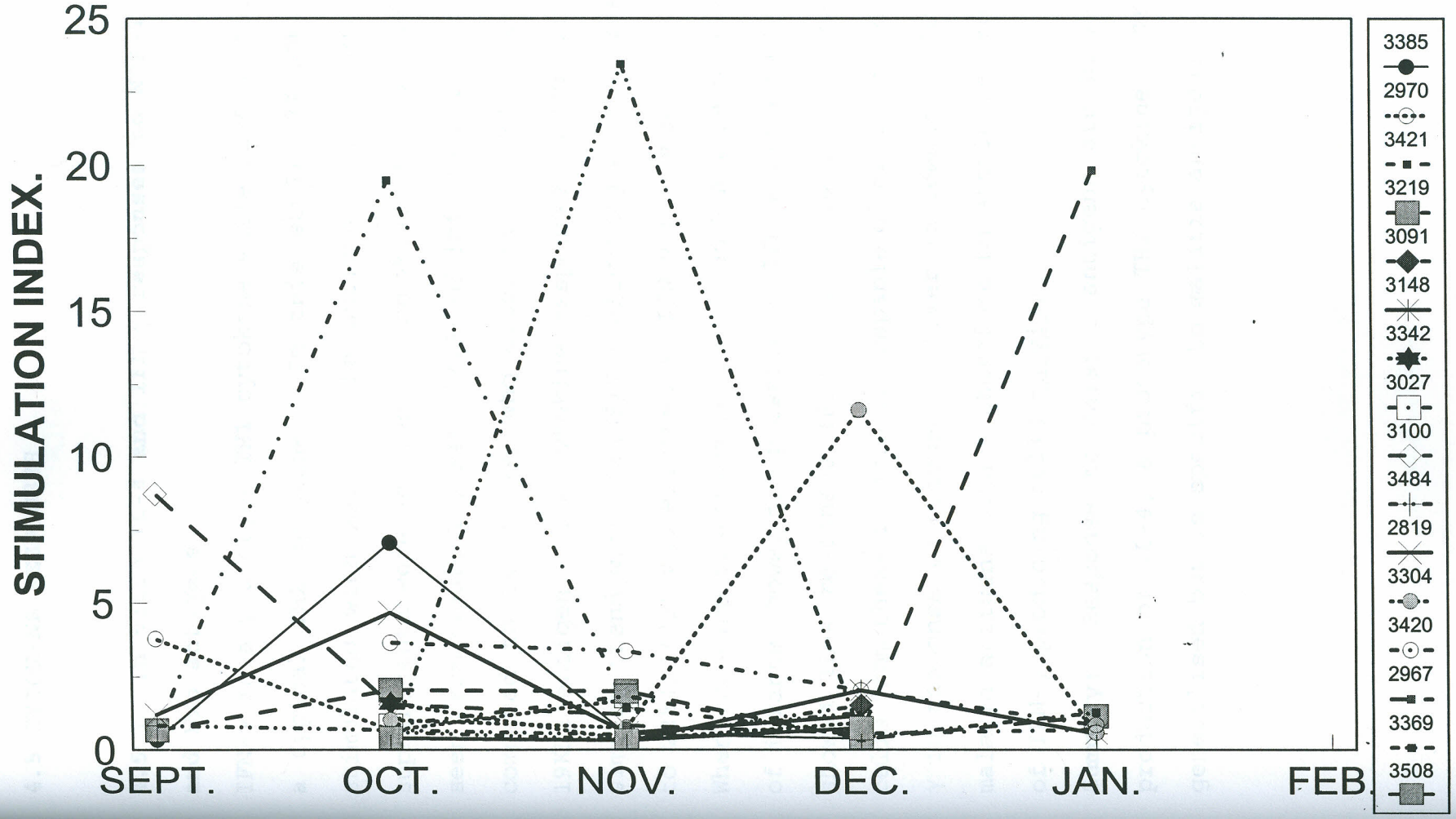


Fig 10
Longitudinal lymphoproliferative responses to exo antigen by PBMCs from infants between September 1995 to February 1996.



4.5 CYTOKINE RESPONSES

4.5.1 In vitro IL-4 and IFN-γ responses to MSP-1 19 kDa antigen and Exo antigens

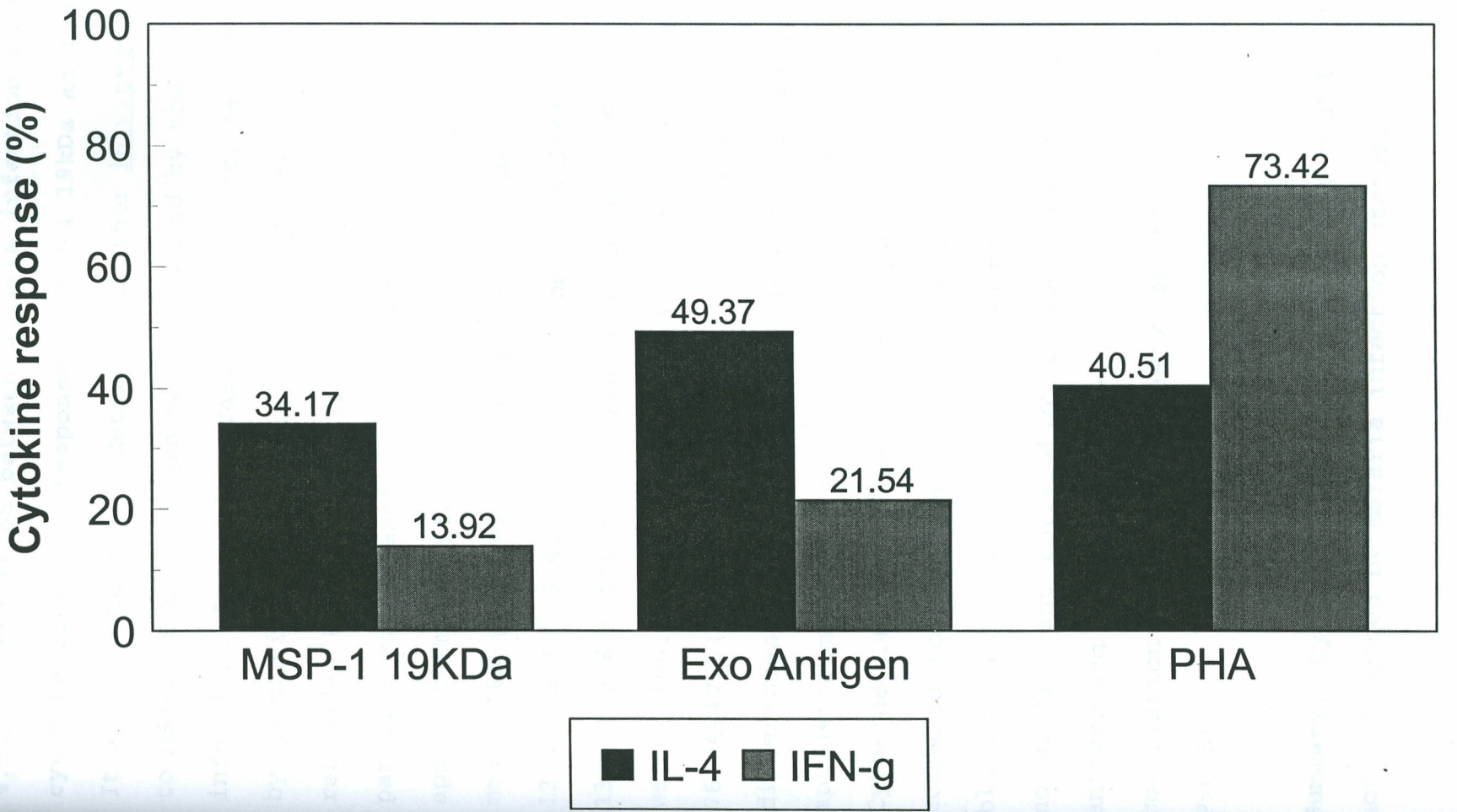
IFN-γ is a prototype TH1 cytokine while IL-4 on the other hand is a typical TH2 cytokine. In this study, 79 PBMC cultures were stimulated with MSP-1 19kDa antigen, exo antigens and PHA and supernatants were assayed for cytokines IL-4 and IFN-γ. As can be seen from figure 11, 34%(27/79) of infants elicited IL-4 responses compared to 14%(11/79) who elicited an IFNγ response to MSP-1 19KDa antigen. The cytokine responses to Exo antigens showed a similar trend with 49%(39/79) eliciting an IL-4 response compared to 22%(17/79) who elicited an IFN-γ response.

When cytokine responses to a mitogen PHA were measured, 73%(63/79) of infants showed the presence of IFN-γ compared to 41%(32/79) who showed IL-4 responses (Fig 11). This finding indicates that T cells from these infants are capable of eliciting an effective IFN-γ in response to mitogens. Lower incidence of IFN-γ response to malaria antigens cannot therefore be attributed to a general lack of IFN-γ producing cells <u>in vivo</u>

Summary: Response to malaria antigens are biased more towards production of IL-4, a prototype TH2 cytokine. This bias is not generalised but is specific to malaria antigens.

Figure 11

***In vitro* IL-4 and IFN- gamma production by PBMCs from infants in response to malaria antigens**



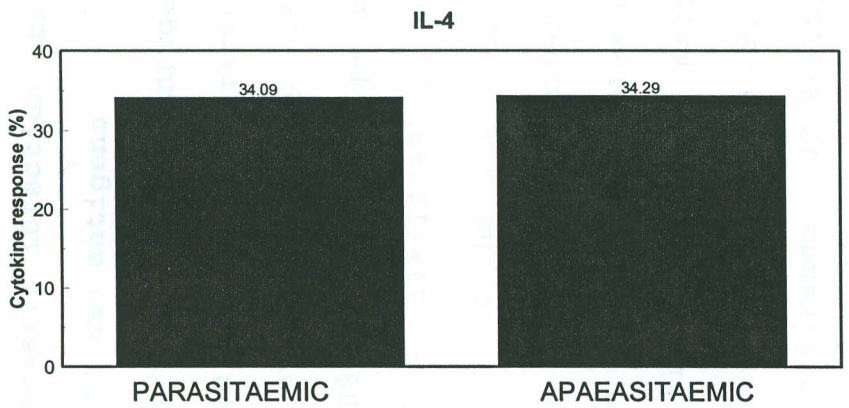
4.5.2 Correlation between malaria infection status and in vitro cytokine levels in response to MSP-1 19kDa antigen

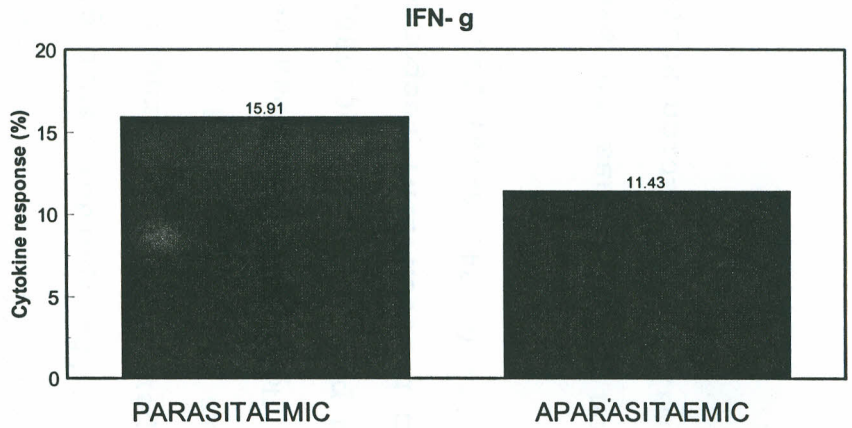
It was of interest to determine whether in vitro cytokine responses to MSP-1 19 kDa antigen was influenced by the prevailing status of infection. Among the parasitemic infants, 34.09% (15/44) responded by producing IL-4 while 34.29% (12/35) of aparasitaemic also responded by producing IL-4. Similarly, while 16% (7/44)of the parasitaemic infants responded by producing IFN-γ 11% (4/35) of the aparasitaemic infants responded by producing IFN-γ (Fig 12). The mean IFN-γ level in response to MSP-1 19kDa antigen was 13.19±13.60(-0.41-26.79) for the parasitemic and 18.11±31.56(-13.45-49.67) for the aparasitemic groups. Those of IL-4 responses were 106.95±81.36(25.59-188.31) for the parasitemic and 76.94±47.1(29.84-124.04) for the aparasitemic groups. The differences in mean cytokine levels between parasitaemics and aparasitaemics were therefore found to be minimal.

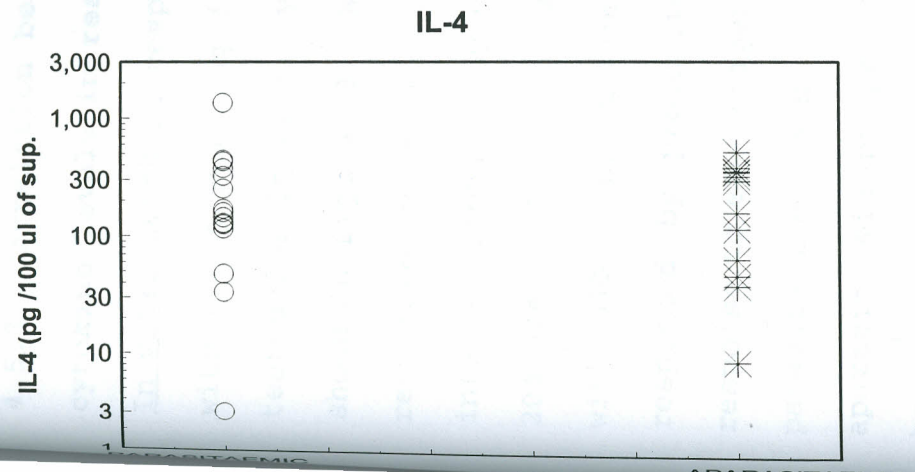
Cytokine concentrations in picograms per 100ul of supernatant were compared to the respective parasite densities per microlitre of blood to determine any relationships. It was found that there was no significant correlation between IL-4 responses to MSP-119kDa antigen and parasitaemia ($r=0.054$, $n=72$; $P>0.5$). There was also no correlation between IFN-γ responses and parasitemia ($r=0.012$, $n=72$; $P>0.5$).

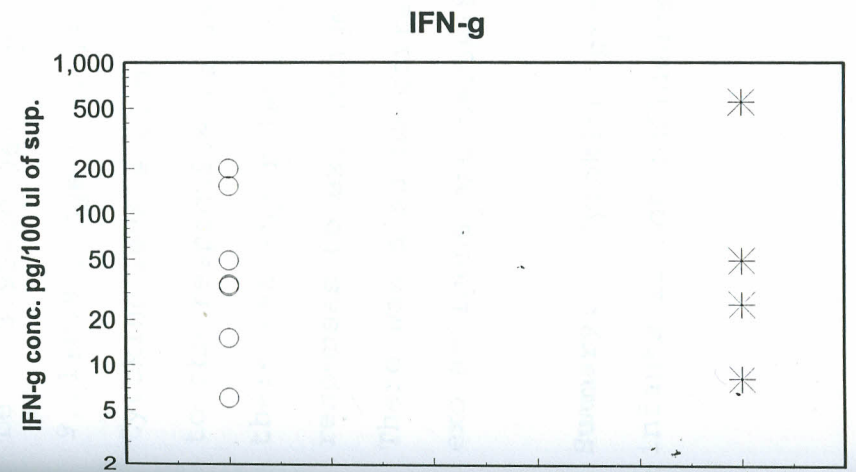
Summary: Cytokine production in response to MSP-1 19KDa antigen is not influenced by malaria infection status.

Fig 12
Relationship between malaria infection status and *in vitro* cytokine levels in response to MSP-1 19 kDa









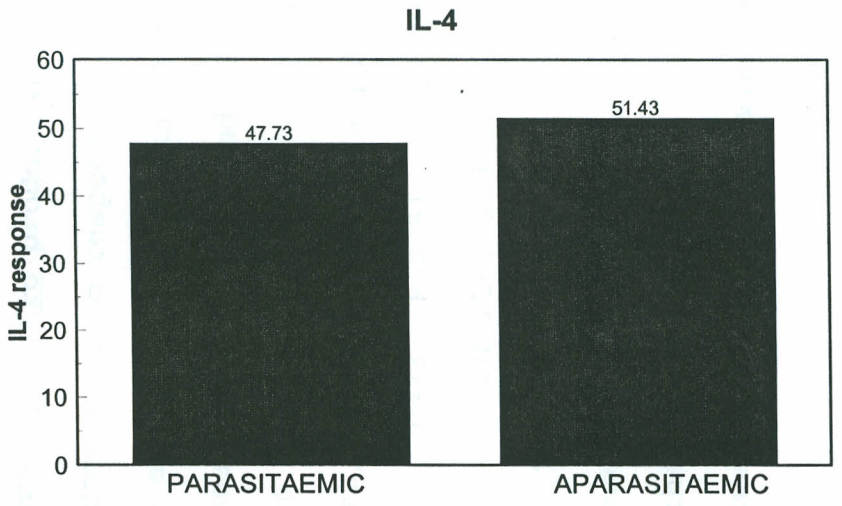
4.5.3 Correlation between malaria infection status and in vitro cytokine levels in response to Exo antigens

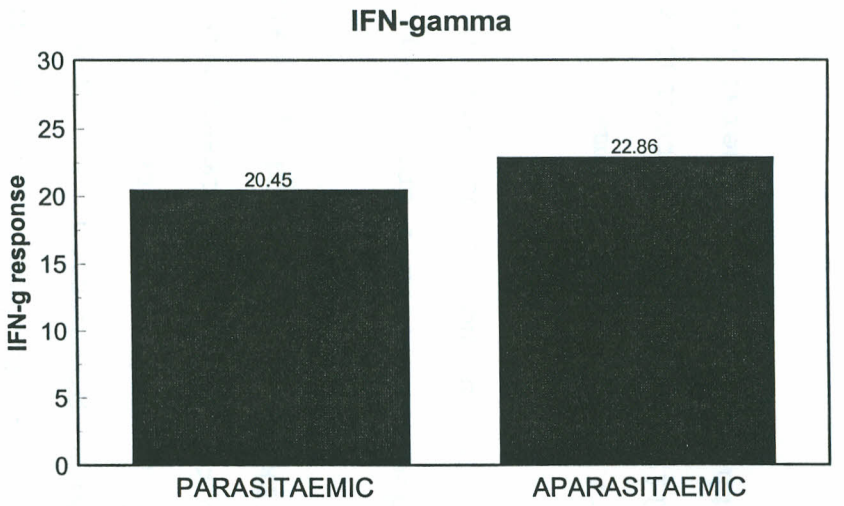
In vitro cytokine responses by PBMCs to exo antigens were compared with the prevailing infection status. The number of incidences tested for in vitro cytokine responses was 79 and the results are shown in figure 13. While 48%(21/44) of the parasitaemic infants responded by production of IL-4, 51%(18/35) of the aparasitaemic infants responded by production of the same. On the other hand, 20%(9/44) of the parasitaemic children responded to exo antigens with IFN-γ production while 23%(8/35) aparasitaemic children responded by production of the same. The mean IL4 level in response to exo antigen was 263.33±193.6(range 69.7-459.6) for the parasitaemic and 205.2±125.4(range 79.8-330.6) for the aparasitaemic groups. On the other hand, that of IFNγ was found to be 28.95±30.96(range -2.01-59.91)for the parasitaemic and 9.31±8.8(range 0.51-18.11) for the aparasitaemic groups.

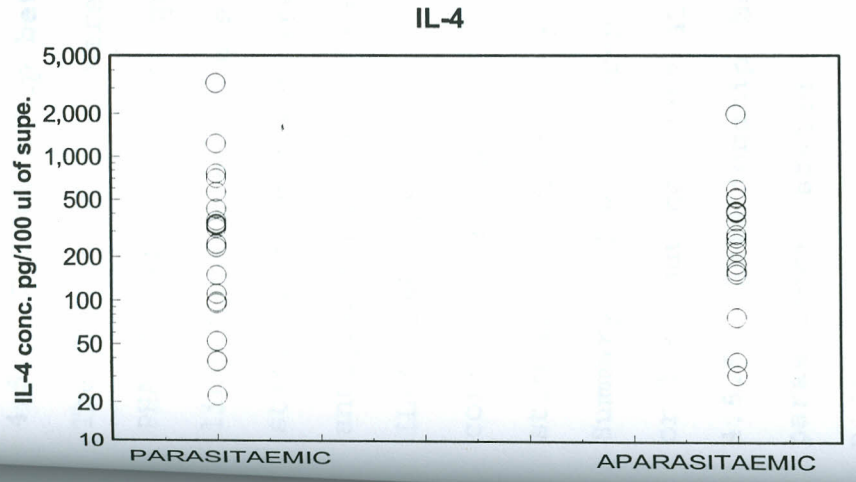
Cytokine levels in picograms per 100ul of supernatant were compared to the respective parasitemias of the individuals to determine if there was any relationship. No correlation was seen between IL4 responses to exo antigens and parasitemia ($r=0.080$, $n=71$; $P>0.5$). There was also no correlation between IFNγ responses of PBMCs to exo antigens and parasitemia ($r=-0.074$, $n=70$; $P>5$).

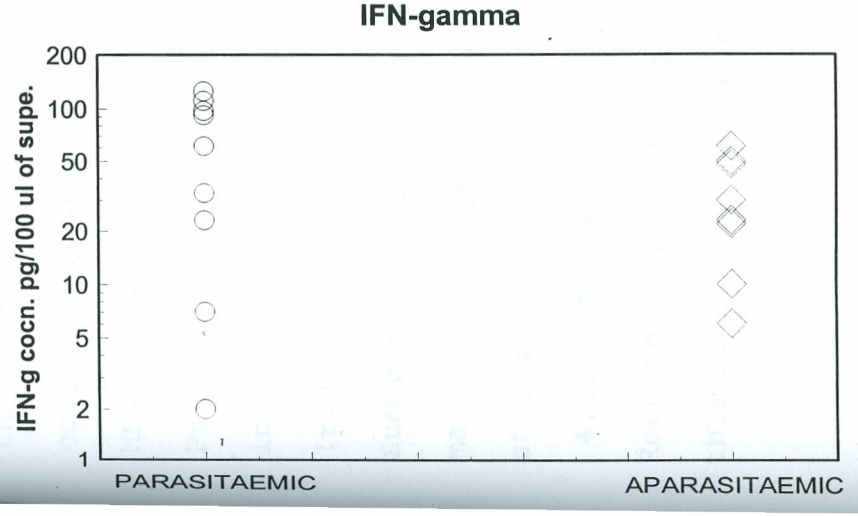
Summary: Cytokine production in response to exo antigens in infants is not influenced by malaria infection status.

Fig 13
Relationship between malaria infection status and cytokine response to exo antigens









4.5.4 Relationship between <u>in vitro</u> production of IFNγ and IL-4

The levels of IL-4 were commpared to respective levels of IFN-γ in PHA, MSP-1 19 KDa antigen and Exo antigens stimulated cultures. No linear correlation was seen between IL-4 and IFN-γ levels in PHA stimulated cultures ($r=-0.146$, $n=73$; $P<0.5$). In the MSP-1 19kDa antigen stimulted cultures, no linear correlation was seen between IL-4 and IFN-γ ($r=-0.076$, $n=73$; $P>0.5$). Likewise, no linear correlation was observed between IL-4 and IFN-γ in Exo antigens stimulated cultures ($r=-0.129$, $n=73$; $P<0.5$).

Summary: The children tested in this study produced either IFN-γ or IL-4 but not both at the same time.

4.5.5 Relationship between <u>in vitro</u> cytokine production and parasitaemia status

Different IL-4 and IFN-γ concentrations were compared with their their respective parasite densities. No significant linear correlation was seen between production of IFN-γ in response to MSP-1 19kDa antigen and parasitaemia status ($r=-0.080$, $n=72$; $P>0.5$). There was also no correlaation between production of IL-4 in response to MSP-1 19kDa antigen and parasitaemia status ($r=0.015$, $n=72$; $P>0.5$).

Summary: The in vitro production of IL-4 and IFN-γ in response to malaria antigen stimulation is not dependent on parasitaemia status.

4.5.6 Prevalence of IFN-γ and IL-4 in plasma

Results from <u>in vitro</u> proliferative and cytokine experiments in this study have suggested that there is a general bias towards TH2

cytokine production in infants. It was of interest therefore to determine IFN-γ and IL-4 levels in plasma and compare this with parasitemia to see if there was any correlation. As shown in figure 14, 83.33%(135/162) of infants showed detectable levels of IL-4 while only 4.3%(7/162) showed detectable IFN-γ (Fig 14) . The mean IL-4 concentration was 38.67 pg/100 ul of plasma while the mean IFN-γ concentration was 12.65 pg/100 ul of plasma. This result complements those of in vitro proliferative and cytokine responses to malaria antigens.

The prevalence of cytokine response in parasitaemic and aparasitaemic groups were compared. Figure 15 shows that 81%(79/98) of parasitemic infants showed the presence of IL-4 while 87.5%(56/64) of the aparasitemic infants showed the presence of the same. On the other hand, 5%(5/98) of parasitemic infants showed the presence of IFN-γ compared to 3.13%(2/64) of aparasitemic infants who showed the presence of the same.

The results show that cytokine levels did not seem to differ between the parasitemic and the aparasitemic groups. Since only very few infants showed detectable IFN-γ in plasma , a meaningful comparison was not possible.

Summary: A majority of infants tested in this study showed prevalence of IL-4 response while a few had detectable IFN-γ response. The presence of IL-4 or IFN-γ was not influenced by clinical status of malaria.

Fig 14
Detectable levelsof IFN -gamma and IL-4 cytokines in plasma of infants in this study

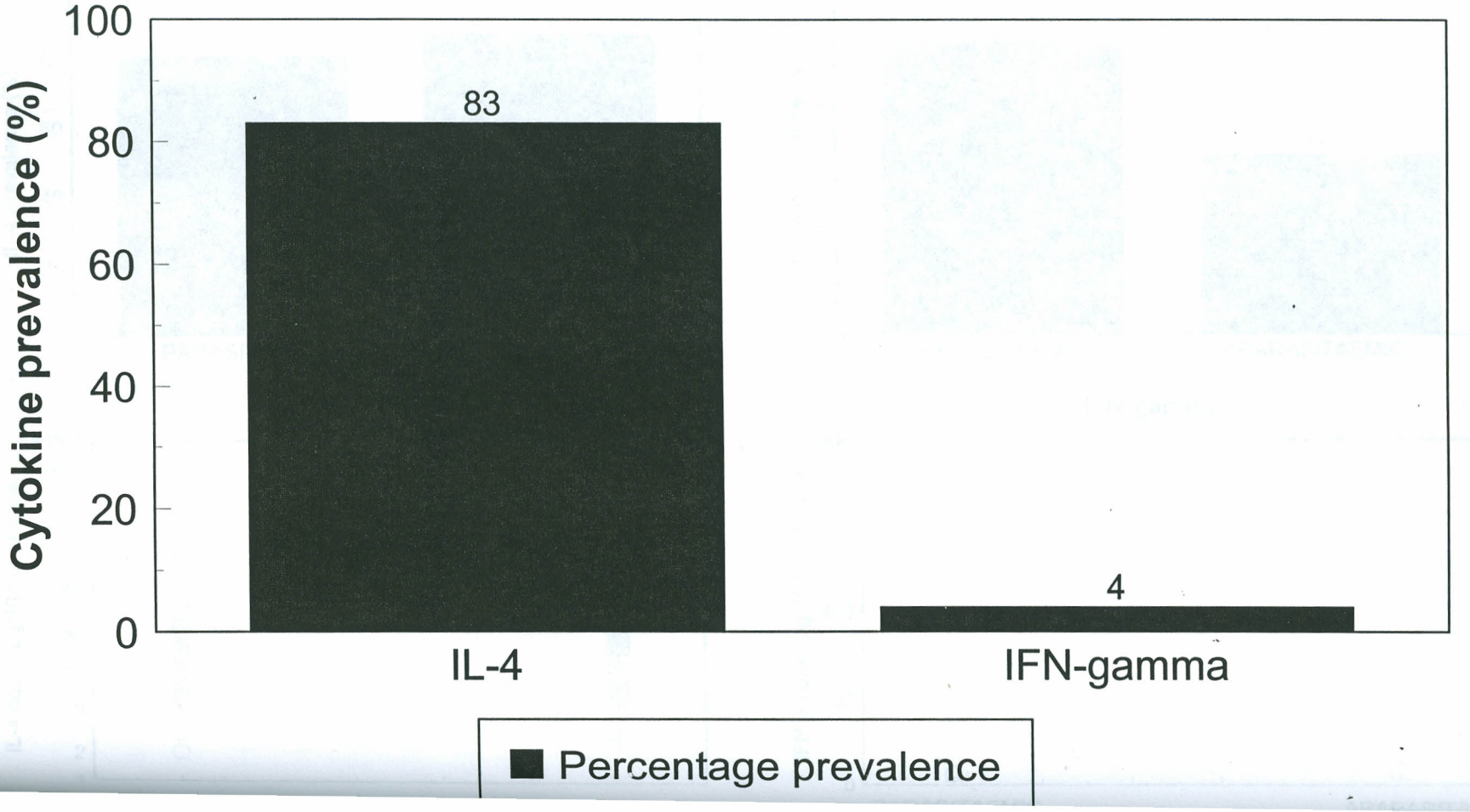
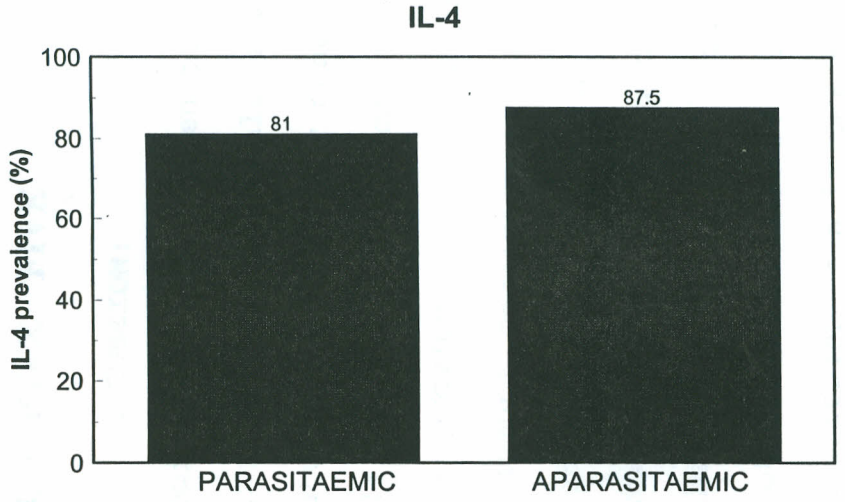
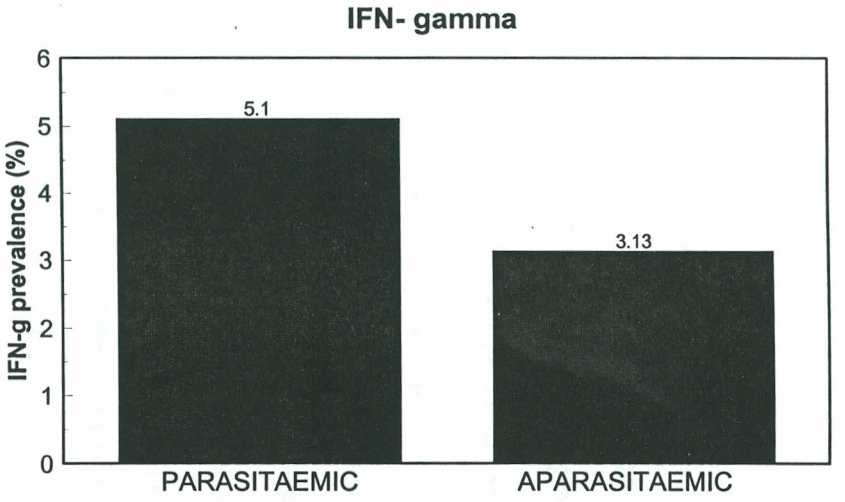
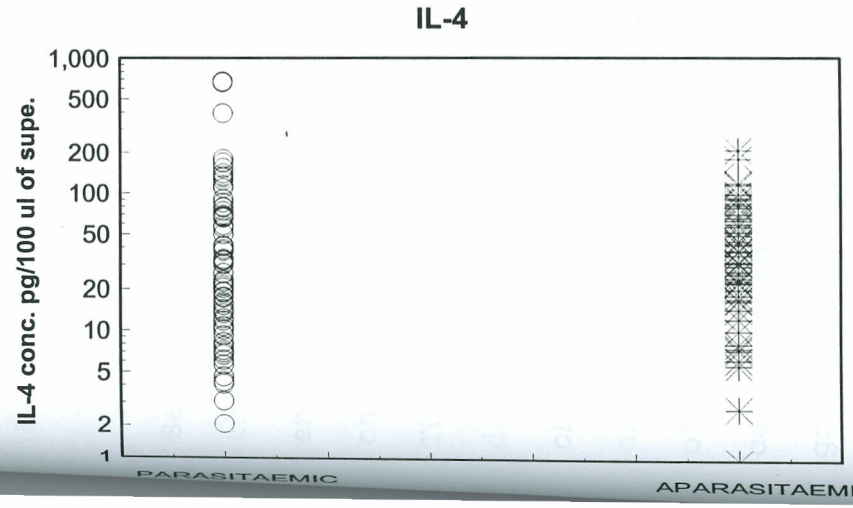
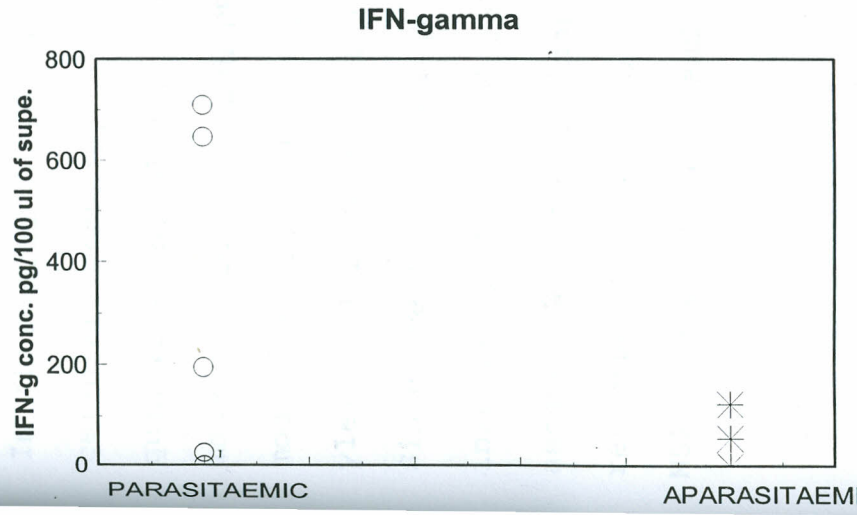


Fig 15
Relationship between malaria infection status and prevalence of plasma cytokine levels









CHAPTER FIVE

DISCUSSION:

Several immuno epidemiologic studies have been conducted to adress the cellular immune responses in individuals living in malaria endemic regions. These studies were however done using adults or children who were hospitalised during a clinical attack of malaria. These studies could only provide limited information on the developement of natural immune mechanisms especially in young children. It was, therefore, important to study the developement of natural immune mechanisms in children during the natural course of malaria. This would provide further insight into the developement of natural protective mechanisms. To accomplish this goal, a sub-set of infants enrolled in a larger cohort project at the Asembo Bay were followed up monthly for 4-7 months.

In this study, one of the practical difficulties in measuring cellular immunity in infants was how to obtain sufficient quantities of blood. To circumvent this problem, an attempt was made to recover enough PBMCs from finger prick blood by a slightly modified Ficoll-hypaque separation. This method consistently yielded $3.10 \pm 0.76 (2.34\text{-}3.86)$ PBMCs per ml of finger prick blood. Since cell numbers were limited, I used 5.0×10^4 cells per well instead of 2.0×10^4 cells normally used in most proliferation assays. With these minor modifications, it was possible to monitor responses to one mitogenic stimuli (PHA) and two malaria antigens; MSP-1 19 kDa antigen and exo antigens. It was found that more than

95% Of the individuals showed positive proliferative responses to PHA using this modified technique. Thus a modified protocol has been developed to successfully monitor cellular immune responses even in infants using finger prick blood.

Previous studies have shown that different parametres of the cell mediated immunity such as proliferation and cytokine responses need to be measured in order to get a more comprehensive picture of cellular immune responses. Since antibody levels to MSP-1 antigen was found to correlate with clinical protection in infants , an attempt was made to determine the cellular immune responses of infants to this antigen.

The MSP-1 19KDa antigen has been shown to be a promising vaccine candidate antigen for malaria (Oaks et al., 1991). Previous studies conducted in the same study area of Asembo bay revealed that individuals exposed to malaria show cellular immune responses to MSP-1 19KDa antigen or peptides representing this antigen (Udhayakumar et al., 1995; Shi et al., 1996). In this study, it has been shown that young children elicit both proliferative and cytokine responses to MSP-1 19KDa antigen. About 15% of infants showed positive proliferative responses (S.I.>2) compared to 35% (Shi et al, 1996) and 30% (Udhayakumar et al, 1995) of adults and higher age groups of children tested in earlier studies. The above studies were carried out in individuals >5 years of age while this study involed infants <2 yaers of age. This suggests that cellular

immunity to MSP-1 19kDa antigen may not be fully developed in these infants.

Longitudinal follow up of immune responses indicated that the proliferative responses were not long lasting. This suggests that natural exposure to the malaria parasite does not lead to the developement of effective memory response in young children. These results are consistent with previous studies that showed that antibody responses to MSP-1 19KDa antigen were short lived (Fruh et al, 1991; Tolle et al., 1993).

Although in some studies, active P.falciparum was shown to cause immunosupression, results of this study showed no difference in the lymphoproliferative responses between the parasitaemic and the aparasitaemic groups.

Laboratory studies suggest that some of the clinical symptoms of malaria such as fever result from the production of parasite derived (exo-) antigens which are liberated into the blood at schizogony. In this study, exo antigens were obtained from parasite culture supernatants and preserved at -20°C until they were used.

While the lymphoproliferative responses to these exo antigens were low in some children it was found to be high in others. This is contrary to the findings of an experiment done using Gabonese children which showed that lymphoproliferative responses were low in all children (Luty et al., 1994). The respose showed no difference between the parasitaemic and the aparasitaemic groups in

this study. On the contrary, Luty et al (1994) have shown that proliferative responses were lower in the group with higher parasitaemia as compared to those with no or lower parasitaemia. Longitudinal follow up indicated that the proliferative responses were not long lasting. The rise and decline of these response suggests a lack of effective memory response to malaria antigen in young children.

In some human parasitic and other disease situations, a polarization of cytokine responses to TH1 or TH2 type has been shown. It is not clear however whether a similar polarization occurs in malaria. The findings of the study described here indicate that a majority of the children showed IL-4 and not IFN-γ responses to both MSP-1 19kDa antigen and soluble exo antigens. However, it was also shown that there was a strong IFN-γ response to PHA in the same children. This therefore indicates that the higher prevalence of IL-4 is not due to a general deficit in IFN-γ production or lack of IFN-γ producing cells in these children. It therefore appears that this preference for IL-4 production is specific to stimulation with malaria antigens. Longitudinal measures of cytokine production also confirmed that indeed most of the individuals responded with the same pattern of cytokine production of IL-4 or IFN-γ at most of the time points tested. The preferential IL-4 response to malaria antigen in these children is consistent with some of the rodent malaria studies that showed that production of IL-4 but not IFN-γ is associated with malaria

antigens (Troye-Blomberg et al., 1990). Likewise, the findings of this study are consistent with those of other studies that suggest that IFN-γ responses are low and do not correlate with proliferative or antibody responses and therefore may not be effective at controlling infection (Riley et al., 1991; Luty et al., 1994)

Althogh the results of this study suggest that cytokine responses to malaria antigens are biased towards a TH2 type of response in children, further studies in different age groups and various endemic settings need to be conducted before making any generalized conclusions.

Determination of plasma cytokine levels revealed that most children showed detectable IL-4 levels. On the contrary, IFNγ levels were barely present. This pattern of plasma cytokine response correlates with in vitro cytokine responses. It is, however, not clear whether this cytokine profile in plasma is soley due to malaria. Lack of correlation between IL-4 and IFN-γ levels in parasitaemic versus aparasitaemic groups suggest that IL-4 and IFN-γ may not be used as reliable markers for the prediction of malaria infection in young children.

CHAPTER SIX

SUMMARY OF CONCLUSIONS

1. An in vitro method using finger prick blood from infants was developed to asses the cellular immune responses to malaria antigens.

2. The magnitude of response to PHA was not influenced by the malaria infection status.

3. Infants responded to MSP-1 19 kDa antigen by proliferation. The responses were low in infants aged between six months to two years compared to the responses in children of more than five years of age and adults in conformity with earlier reports.

4. Proliferative responses to both MSP-1 19KDa and exo-antigens were short lived and did not correlate with parasitaemia.

5. The in vitro cytokine production in response to malaria antigens showed a bias towards production of IL-4, a prototype TH2 cytokine.

6. Cytokine production to both MSP-1 19KDa antigen and exo antigens did not correlate with malaria infection status.

7. Most of the infants living in this high malaria endemic area showed a higher prevalence in plasma of IL-4 than IFN-γ.

CHAPTER SEVEN

PROJECTIONS FOR FUTURE STUDIES

1. Longitudinal follow up of natural immune responses should be conducted at various other endemic settings.

2. Children should be followed up to 5 years of age in order to assess fully the development of natural immune responses.

3. Longitudinal humoral immune responses should be part of the study in order to assess any correlation between proliferation /cytokine responses and antibody responses.

4. Other TH1/TH2 prototype cytokines like IL-2(TH1) and IL10(TH2) should be assayed in young children longitudinally.

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APPENDICES

APPENDIX I

RECIPE OF REAGENTS:

Culture medium:

A mixture of RPMI-1640 medium (GIBCO-BRL, Grand Island, NY), 5% normal AB+ human serum (donors not previously exposed to malaria), 5% Fetal Bovine serum and 2mM glutamine, 100U/ml and streptomycin was used.

Antigens:

MSP-1 19 kDa antigen (C-terminal) was made in the Biotechnology Core Facility of National Center for Infectious Diseases at Centers for Disease Control and Prevention, Atlanta, Georgia. This antigen was more than 90% pure by HPLC analysis and was used without further purification.

Exo antigens were obtained by collection of P. falciparum culture supernatant at about day 4 of culture and storing at 20°C untill when ready for use.

NFANTS IDENTIFICATION

VILLAGE	SAMPLE	D.O.B.	AGE(Yrs)	#OF TIMES FOLLOWED
VILL. 1	3056	26.03.94	1.9	3
	3385	17.01.95	1.1	5
	3290	20.09.94	1.4	2
	3081	27.05.94	-----	3
	3050	06.01.94	2.1	2
	3524	13.06.95	0.7	4
	2767	09.09.93	2.4	3
VILL. 2	SAMPLE	D.O.B.	AGE(Yrs)	# OF TIMES FOLLOWED
	3036	01.04.94	1.9	5
	2970	26.03.94	1.9	5
	3480	18.01.95	1.1	3
	3404	25.01.95	1.1	5
	3034	30.04.94	1.9	4
	3529	04.05.95	0.8	1
VILL. 3	SAMPLE	D.O.B	AGE(Yrs)	# OF TIMES FOLLOWED
	3001	26.02.94	2	4
	3561		-----	2
	2821	21.01.94	2.1	4
	3464	13.12.94	1.2	1
	3513	20.01.95	DIED	1
VILL. 4	SAMPLE	D.O.B.	AGE(Yrs)	# OF TIMES FOLLOWED
	2934	03.04.94	1.9	4
	2927	01.02.94	2	5
	3421	06.01.95	1.2	5
	3219	11.11.94	1.3	7
	3091	27.03.94	1.9	6
	3411	28.01.95	1.1	4
	3254	04.08.94	1.5	4
	2844	17.02.94	2	2
	3199	14.04.94	1.9	4
VILL. 5	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED
	2862	10.02.94	2	3
	2936	30.04.94	1.9	4
	3476	24.11.94	1.3	1
	3148	13.08.94	1.5	5
VILL. 6	SAMPLE	D.O.B.	AGE	# OF TIMMES FOLLOWED
	3020	15.03.94	1.9	-----
	3463	20.01.95	1.1	5

	3468	23.01.95	1.1	2			
	3397	13.11.94	1.2	4			
	3019	19.02.94	2	4			
VILL. 7	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	3342	25.11.94	1.2	5			
	3399	21.12.94	1.2	5			
	3339		-----	2			
	3336	13.02.95	1	2			
VILL. 8	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	3142	03.04.94	1.9	4			
	3027	17.04.94	1.9	5			
	3401	21.12.94	1.2	7			
	3429	23.11.94	1.3	4			
VILL. 9	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	2955	05.02.94	2	3			
	3104	05.02.94	2	-----			
VILL. 10	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	3100	10.02.94	2	5			
	3344	23.10.94	1.4	5			
	3761	24.08.95	0.5	4			
VILL. 11	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	2998	05.04.94	2	2			
	3492	20.01.95	1.2	3			
	3359	01.10.94	2.1	3			
	3293	12.09.94	1.4	3			
	3599	12.04.95	2	2			
	3770	22.08.95	0.5	4			
	2513	27.04.93	3	4			
	2672	06.06.93	2.7	2			
VILL. 12	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	3440	24.02.95	2	5			
	3484	27.01.95	1.1	6			
	2819	27.01.94	2.1	6			
	2901	10.02.94	2	4			
	3116	15.08.94	1.5	3			
	3220	09.08.94	1.5	2			
	3443	02.08.95	0.5	1			

INFANTS IDENTIFICATION

	2832	30.11.93	2.3	3			
	2875	03.03.94	2	2			
VILL. 13	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	2835	25.02.94	1.9	5			
	2859	04.02.94	1.9	1			
	2974	12.01.94	2.2	3			
	3304	04.01.95	1.1	5			
	3420	19.12.94	1.2	6			
	3306	03.11.94	1.3	4			
	3729		-----	2			
	3509	03.04.95	0.9	2			
	3627	18.07.95	0.6	2			
	3727	01.07.95	0.6	2			
	3298		-----	1			
	2633	02.04.93	2.9	2			
VILL. 14	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	3006	06.03.94	1.9	7			
	2967	25.04.94	1.9	5			
	3356	13.11.94	1.2	6			
	3369	05.12.94	1.2	7			
	3435	04.10.94	1.3	6			
	3265	04.10.94	1.3	5			
	3508	25.05.95	0.8	6			
	3730	22.08.95	0.5	4			
VILL. 15	SAMPLE	D.O.B.	AGE	# OF TIMES FOLLOWED			
	3052	23.02.94	2	4			
	3242	11.05.94	1.9	1			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

3385	T1	T2	T3	T4	T5		
DATE	19.9.95	19.10.95	16.11.95	5.12.95	31.1.96		
PHA-SI	19.97	5.47	6.79	13.05	45.03		
EKNG-SI	0.38	0.76	1.17	0.88	0.94		
EXO-N-SI	0.35	7.06	0.54	0.38	-----		
TEMP	36.2	36.3	36.1	38.3	38.3		
HB	14.3	15.1	13.6	13.5	ND		
PAR.D/ml	1946.67	0	0	97066.67	0		
#CELLS	100,000	100,000	100,000	100,000	50,000		
3524	T1	T2	T3	T4			
DATE	17.11.95	12.1.96	8.2.96	7.3.96			
PHA-SI	7.75	22.52	4.29	12.25			
EKNG-SI	1.29	2.05	0.71	0.72			
EXO-N-SI	1.65	2.53	-----	-----			
TEMP	36.3	36.8	36.8				
HB	11.5	ND	ND	ND			
PAR.D/ml	0	0	0	0			
#CELLS	100,000	100,000	100,000	100,000			
3036	T1	T2	T3	T4	T5		
DATE	17.10.95	14.11.95	14.12.95	10.1.96	8.2.96		
PHA-SI	7.24	9.85	120.31	4.86	12.7		
EKNG-SI	0.48	1.23	1.38	0.42	0.61		
EXO-N	0.63	1.52	3.95	0.49	-----		
TEMP	36.6	36.1	36		36.7		
HB	9.9	11.7	8.9	13.6	ND		
PAR.D/ml	1600	4826.67	1680	2506.67	2506.67		
#CELLS	50,000	50,000	50,000	100,000	50,000		
2970	T1	T2	T3	T4	T5		
DATE	19.10.95	17.11.95	14.12.95	31.1.96	15.2.96		
PHA-SI	2.44	5.05	13.59	3.14	23.52		
E-KNG-SI	0.46	0.43	0.89	0.73	2.6		
EXO-N-SI	3.78	0.67	1.71	-----	-----		
TEMP	38.1	36.2	36.3	36.9	37		
HB	8.1	12	14.7	ND	ND		
PAR.D/ml	0	2000	0	1653.33	0		
#CELLS	50,000	50,000	100,000	100,000	100,000		
3404	T1	T2	T3	T4	T5		
DATE	19.9.95	19.10.95	14.11.95	18.1.96	15.2.96		
PHA-SI	31.24	4.62	18.31	32.34	4.15		

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

E-KNG-SI	1.15	0.58	1.61	1.12	0.54		
EXO-N-SI	0.54	7.43	0.85	-----	-----		
TEMP	36.4	36.8	37	38.8	36.8		
HB	10.3	13.6	11.6	ND	ND		
PAR.D/ml	0	0	0	11306.67	0		
#CELLS	100,000	50,000	50,000	40,000	50,000		
3034	T1	T2	T3	T4			
DATE	19.9.95	17.10.95	12.1.96	9.2.96			
PHA-SI	45.78	28.43	13.65	9.11			
E-KNG-SI	1.61	0.96	1.01	5.94			
EXO-N-SI	0.99	21.66	0.7	-----			
TEMP	36.4	36.9	36.3	36.3			
HB	10.6	10.7	ND	ND			
PAR.D/ml	3600	4400	7866.67	133			
#CELLS	32,000	50,000	50,000	100,000			
3001	T1	T2	T3	T4			
DATE	26.9.95	23.11.95	16.1.96	15.2.96			
PHA-SI	3.88	12.35	21.79	2.72			
E-KNG-SI	0.96	1.43	1.84	2.67			
EXO-N-SI	11.54	2.18	-----	-----			
TEMP	36	36					
HB	ND	16.1	ND	ND			
PAR.D/ml	4186.67	1653.33	3333.33	1920			
#CELLS	50,000	100,000	50,000	30,000			
2821	T1	T2	T3	T4			
DATE	16.11.95	12.12.95	23.1.96	20.2.96			
PHA-SI	101.64	23.64	33.79	4.47			
E-KNG-SI	2.81	0.79	0.83	0.22			
EXO-N-SI	7.26	1.05	-----	-----			
TEMP	36.4	36.4	36.7				
HB	10.9	12.1	ND	ND			
PAR.D/ml	7840	6640	4880	11066.67			
#CELLS	100,000	100,000	50,000	40,000.79			
2934	T1	T2	T3	T4			
DATE	19.9.95	14.11.95	7.12.95	20.2.96			
PHA-SI	-----	19.77	12.04	4.87			
E-KNG	-----	0.44	1.6	0.42			
EXO-N-SI	-----	0.4	0.84	-----			
TEMP	37.1	37.6	37.8	36.7			
HB	8.3	8.2	14.7	ND			
PAR.D/ml	0	10426.67	5840	2400			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

#CELLS	100,000	50,000	100,000	100,000			
2927	T1	T2	T3	T4	T5	T6	
DATE	5.10.95	9.10.95	14.11.95	14.12.95	16.1.96	20.2.96	
PHA-SI	40.45	0.74	37.56	74.45	45.75	12.43	
E-KNG	2.89	0.09	2.31	5.68	0.94	0.64	
EXO-N-SI	1.59	1.58	0.76	5.38	-----	-----	
TEMP	36.1	39.4	36	36.9	38.3		
HB	ND	ND	19.7	7.8	ND	ND	
PAR.D/ml	0	133.33	906.67	400	453	1520	
#CELLS	100,000	50,000	50,000	30,000	40,000	50,000	
3421	T1	T2	T3	T4	T5		
DATE	19.9.95	5.10.95	17.10.95	14.11.95	5.12.95		
PHA-SI	23.18	0.42	16.65	7.2	45.75		
E-KNG-SI	2.31	0.26	1.01	0.58	0.94		
EXO-N-SI	0.63	19.46	1.43	0.56	0.7		
TEMP	36.9	39.3	36.7	36.7	38.3		
HB	11.4	14.1	13	12.7	11.5		
PAR.D/ml	5840	1013.33	0	0	453		
#CELLS	100,000	100,000	100,000	50,000	100,000		
3219	T1	T2	T3	T4	T5	T6	T7
DATE	19.9.95	17.10.95	14.11.95	21.11.95	14.12.95	16.1.96	22.2.96
PHA-SI	29.02	17.58	34.09	14.67	18.9	39.55	4.02
E-KNG-SI	1.94	4.23	2.73	0.92	2.2	0.81	1.02
EXO-N-SI	0.68	2.05	2	0.37	1.16	-----	-----
TEMP	36.7	36.4	36.6	38.4	36.2	37.6	-----
HB	14.6	12.6	9.2	13.6	10.2	ND	ND
PAR.D/ml	506.67	2480	0	0	106.67	16240	186.67
#CELLS	100,000	100,000	100,000	100,000	100,000	50,000	100,000
3091	T1	T2	T3	T4	T5	T6	
DATE	19.9.95	17.10.95	14.11.95	14.12.95	23.1.96	22.2.96	
pha-si	-----	11.76	8.81	53.22	32.28	5.66	
e-kng-si	-----	0.76	1.02	1.34	1.03	0.69	
EXO-N-SI	------	0.62	0.36	1.52	-----	-----	
temp	36.5	36.4	37.8	36.3	37.7	-----	
HB	10.7	10.3	17.7	12.1	ND	ND	
par.d/ML	1146.67	0	266.67	0	2773.33	0	
#CELLS	100,000	100,000	35,000	50,000	40,000	50,000	
3411	T1	T2	T3	T4			
DATE	16.11.95	23.1.96	8.2.96	20.2.96			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

PHA-SI	2.72	10.68	10.42	2.57			
E-KNG-SI	0.6	0.72	1.57	0.41			
EXO-N-SI	0.97	-----	-----	-----			
TEMP	37.7	36.6	36.9	-----			
HB	12.2	ND	ND	ND			
PAR.D/ml	5146.67	0	0	6.4			
#CELLS	100,000	100,000	100,000	100,000			
3254	T1	T2	T3	T4			
DATE	16.11.95	24.1.96	6.2.96	6.3.96			
PHA-SI	2.3	5.81	25.9	7.35			
E-KNG-SI	0.42	0.76	0.81	1.24			
EXO-N-SI	0.76	-----	-----	-----			
TEMP	36.7	36.1	38.2	-----			
HB	14.4	ND	ND	ND			
PAR.D/ml	0	9013.33	4693.33	5466.67			
#CELLS	100,000	100,000	50,000	100,000			
3199	T1	T2	T3	T4			
DATE	17.11.95	24.1.96	9.2.96	7.3.96			
PHA-SI	21.01	24.02	6.09	8.82			
E-KNG-SI	0.79	0.27	0.58	0.58			
EXO-N-SI	0.48	-----	-----	-----			
TEMP	36.6	38.7	-----	-----			
HB	14.5	ND	ND	ND			
PAR.D/ml	27573.33	613.33	26.67	3626.67			
#CELLS	50,000	50,000	100,000	100,000			
2936	T1	T2	T3	T4			
DATE	12.10.95	16.1.96	18.1.96	13.2.96			
PHA-SI	9.32	11.21	6.42	62.43			
E-KNG-SI	2.53	4.12	0.7	0.94			
EXO-N	1.34	-----	-----	-----			
TEMP	36.4	37	38.5	36.1			
HB	11	ND	ND	ND			
PAR.D/ml	0	9813.33	0	1760			
#CELLS	100,000	50,000	40,000	50,000			
3148	T1	T2	T3	T4	T5		
DATE	17.11.95	14.12.95	10.1.96	6.2.96	7.3.96		
PHA-SI	5.05	45.9	0.59	93.73	10.26		
E-KNG-SI	0.43	2.8	0.77	1.56	0.61		
EXO-N-SI	0.29	2.03	0.56	-----	-----		
TEMP	37.5	38.8	36.2	37.1	-----		
HB	5.6	16.8	14.5	ND	ND		

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

PAR.D/ml	2000	18960	0	6586.67	0		
#CELLS	100,000	40,000	100,000	50,000	50,000		
3463	T1	T2	T3	T4	T5		
DATE	12.10.95	7.11.95	16.1.96	25.1.96	1.2.96		
PHA-SI	13.29	99.39	18.93	42.96	8.67		
E-KNG-SI	0.71	0.86	0.54	1.08	2.05		
EXO-N-SI	0.62	3.38	------	-----	-----		
TEMP	36.5	35.7	39.6	37.5	39.1		
HB	14.9	16.1	ND	ND	ND		
PAR.D/ml	0	0	0	0	14026.67		
#CELLS	50,000	30,000	40,000	100,000	50,000		
3397	T1	T2	T3	T4			
DATE	16.11.95	14.12.95	18.1.96	6.2.96			
PHA-SI	68.17	16.58	7.03	10.09			
E-KNG-SI	3.5	0.79	0.72	0.58			
EXO-N-SI	1.33	0.33	-----	-----			
TEMP	37.7	36.4	36.9	37.8			
HB	15.3	10.5	ND	ND			
PAR.D/ml	2560	986.67	0	26.67			
#CELLS	100,000	40,000	25,000	50,000			
3019	T1	T2	T3	T4			
DATE	13.10.95	7.11.95	5.12.95	8.2.96			
PHA-SI	22.68	14.36	5.47	26.01			
E-KNG-SI	0.95	0.83	1.63	0.7			
EXO-N-SI	22.24	2.26	1.39	-----			
TEMP	36.8	37.6	36	36.9			
HB	ND	13.9	14.5	ND			
PAR.D/ml	0	0	0	7760			
#CELLS	100,000	50,000	100,000	100,000			
3342	T1	T2	T3	T4	T5		
DATE	12.10.95	7.11.95	5.12.95	24.1.96	15.2.96		
PHA--SI	3.3	1.81	18.59	16.39	3.05		
E-KNG-SI	2.84	0.78	0.86	0.49	0.65		
EXO-N-SI	1.6	0.35	0.93	-----	-----		
TEMP	36.9	37.5	36	38.5	36.7		
HB	6.4	9.5	11	ND	ND		
PAR.D/ml	0	2213.33	0	23066.67	0		
#CELLS	100,000	100,000	100,000	50,000	100,000		
3399	T1	T2	T3	T4	T5		

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

DATE	9.11.95	28.11.95	7.12.95	18.1.96	15.2.96		
PHA-SI	8.88	52.24	19.87	7.07	5.05		
E-KNG-SI	1.18	1.17	1.8	0.72	1.49		
EXO-N-SI	1.51	1.03	1.59	-----	-----		
TEMP	37	39	-----	36.9	37		
HB	9.4	11.9	12.9	ND	ND		
PAR.D/ml	10373.33	22746.67	80	4720	19466.67		
#CELLS	100,000	50,000	100,000	100,000	50,000		
3142	T1	T2	T3	T4			
DATE	7.12.95	16.1.96	18.1.96	13.2.96			
PHA-SI	4.83	23.68	0.36	61.27			
E-KNG-SI	0.88	1.88	1.05	2.03			
EXO-N-SI	0.66	-----	-----	-----			
TEMP	36.4	36.9	36.2	36.8			
HB	12.2	ND	ND	ND			
PAR.D/ml	1360	80	0	10560			
#CELLS	100,000	50,000	100,000	50,000			
3027	T1	T2	T3	T4	T5		
DATE	12.10.95	9.11.95	7.12.95	18.1.96	15.2.96		
PHA-SI	7.97	18.61	6.86	15.37	2.39		
E-KNG-SI	0.44	1	2.63	1.51	0.86		
EXO-N-SI	0.84	1.84	0.41	-----	-----		
TEMP	36.6	36.7	36.6	36.8	36.7		
HB	10.4	14.3	17.6	ND	ND		
PAR.D/ml	0	0	0	826.67	1413.33		
#CELLS	50,000	35,000	100,000	50,000	50,000		
3401	T1	T2	T3	T4	T5	T6	T7
DATE	12.10.95	9.11.95	7.12.95	10.1.96	18.1.96	8.2.96	15.2.96
PHA-SI	29.73	15.24	35.63	2.45	2.36	7.48	6.5
E-KNG-SI	2.15	0.77	1.65	0.7	1.13	0.39	1.13
EXO-N-SI	1.61	0.85	1.96	1.24	-----	-----	------
TEMP	37.6	36.6	37	37.1	37.2	36	36.8
HB	9.8	14	7.7	10.8	ND	ND	ND
PAR.D/ml	53.33	0	213.33	42346.67	853.33	106.67	586
#CELLS	34,000	30,000	50,000	100,000	50,000	50,000	50,000
3429	T1	T2	T3	T4			
DATE	12.10.95	25.10.95	9.11.95	15.2.96			
PHA-SI	7.01	25.49	39.03	15.84			
E-KNG-SI	0.3	0.51	1.85	0.78			
EXO-N-SI	0.86	8.85	2.32	-----			
TEMP	36.8	38.5	36.8	36.5			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

HB	15.1	13.2	12.5	ND			
PAR.D/ml	0	0	0	106.67			
#CELLS	36,000	100,000	30,000	100,000			
3100	T1	T2	T3	T4	T5		
DATE	26.9.95	24.10.95	21.11.95	18.1.96	23.1.96		
PHA-SI	4.71	2.7	38.3	47.32	34.76		
E-KNG-SI	1.84	0.57	1.41	0.51	1		
EXO-N-SI	8.73	1.56	1.21	-----	-----		
TEMP	36.6	36.3	36.3	36.4	36.2		
HB	8.9	10.1	10.8	ND	ND		
PAR.D/ml	4186.67	0	0	2853.33	0		
#CELLS	50,000	50,000	50,000	50,000	40,000		
3344	T1	T2	T3	T4	T5		
DATE	26.9.95	24.10.95	23.11.95	18.1.96	15.2.96		
PHA-SI	27.13	11.79	30.85	58.43	15.43		
E-KNG-SI	0.28	0.89	0.93	2.64	2.45		
EXO-N-SI	13.74	0.96	1.19	-----	-----		
TEMP	36.3	36	36.8	37.7	36.2		
HB	9.7	6.5	15.2	ND	ND		
PAR.D/ml	19013.33	800	13040	4533.33	16106.67		
#CELLS	100,000	100,000	40,000	50,000	50,000		
3761	T1	T2	T3	T4			
DATE	15.11.95	14.12.95	6.2.96	6.3.96			
PHA-SI	23.3	28.11	18.15	45.11			
E-KNG-SI	0.78	0.53	0.72	0.89			
EXO-N-SI	0.48	1.51	-----	-----			
TEMP	36.2	36.5	36.2	-----			
HB	14.2	10.2	ND	ND			
PAR.D/ml	0	41920	826.67	46906.67			
#CELLS	50,000	50,000	50,000	50,000			
3770	T1	T2	T3	T4			
DATE	16.11.95	12.12.95	16.1.96	13.2.96			
PHA-SI	5.01	31.32	7.61	31.3			
E-KNG-SI	0.79	1.12	1.22	0.95			
EXO-N-SI	0.42	0.51	-----	-----			
TEMP	36.5	36.7	36.6	36.5			
HB	13	13.4	ND	ND			
PAR.D/ml	0	29546.67	12800	13226.67			
#CELLS	100,000	50,000	100,000	50,000			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

2513	T1	T2	T3	T4			
DATE	16.11.95	12.12.95	16.1.96	13.2.96			
PHA-SI	38.6	62.44	2.33	41.6			
E-KNG-SI	1.05	1.84	1.21	0.77			
EXO-N-SI	0.88	0.98	-----	-----			
TEMP	37.2	36.1	36.4	36.4			
HB	21.9	15.1	ND	ND			
PAR.D/ml	7360	293.33	3600	4453.33			
#CELLS	100,000	100,000	100,000	40,000			
3440	T1	T2	T3	T4	T5		
DATE	19.10.95	14.11.95	18.1.96	25.1.96	22.2.96		
PHA-SI	63.13	6.82	6.92	7.91	0.55		
E-KNG-SI	1.07	2.01	0.9	0.7	0.14		
EXO-N-SI	24	0.91	-----	-----	-----		
TEMP	36.3	36.5	36.3	36.7	36.3		
HB	16.1	10.2	ND	ND	ND		
PAR.D/ml	986.67	4586.67	13093.33	746.67	3760		
#CELLS	100,000	100,000	50,000	40,000	50,000		
3484	T1	T2	T3	T4	T5	T6	
DATE	21.9.95	16.11.95	21.11.95	18.1.96	25.1.96	22.2.96	
PHA-SI	27.71	4.23	18.17	16.71	9.19	15.21	
E-KNG-SI	2.62	0.81	0.64	1.27	1.02	1.21	
EXO-N-SI	0.84	0.52	1.28	-----	-----	-----	
TEMP	36.7	36.7	39.4	36.4	36.4	36.7	
HB	9.5	8.7	12.6	ND	ND	ND	
PAR.D/ml	53.33	1253.33	0	0	0	4906.67	
#CELLS	100,000	100,000	50,000	50,000	40,000	100,000	
2819	T1	T2	T3	T4	T5	T6	
DATE	21.9.95	19.10.95	16.11.95	12.12.95	25.1.96	22.2.96	
PHA-SI	58.61	8.46	27.63	243.33	1.95	0.45	
E-KNG-SI	0	0.42	1.31	0.99	0.98	0.99	
EXO-N-SI	1.22	4.67	0.58	1.17	-----	-----	
TEMP	36	36	37	38.1	36	36.8	
HB	12.2	10.5	15.4	13.6	ND	ND	
PAR.D/ml	3093.33	0	0	5520	426.67	1893.33	
#CELLS	100,000	50,000	50,000	100,000	40,000	50,000	
2901	T1	T2	T3	T4			
DATE	21.9.95	19.10.95	16.11.95	25.1.96			
PHA-SI	100	27.04	6.64	31.68			
E-KNG-SI	0.6	0.67	0.26	3.63			
EXO-N-SI	0.52	6.03	0.22	-----			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

TEMP	36.4	36.9	36	36.3			
HB	14	ND	6.3	ND			
PAR.D/ml	906.67	453.33	3066.67	5813.33			
#CELLS	100,000	100,000	100,000	40,000			
2835	T1	T2	T3	T4			
DATE	5.10.95	31.10.95	6.2.96	5.3.96			
PHA-SI	14.37	5.83	7.93	8.16			
E-KNG-SI	1.05	0.64	2.48	0.36			
EXO-N-SI	24.81	1.91	----	-----			
TEMP	36	36	36.6	35.8			
HB	12.8	10.2	ND	ND			
PAR.D/ml	11920	2746.67	426.67	4826.67			
#CELLS	50,000	50,000	50,000	50,000			
3304	T1	T2	T3	T4	T5		
DATE	5.10.95	2.11.95	28.11.95	11.1.96	5.3.96		
PHA-SI	2.72	0.51	15.55	1.28	9.98		
E-KNG-SI	1	0.63	1.21	1.82	2.46		
EXO-N-SI	1.03	0.76	11.62	0.59	-----		
TEMP	36.5	36	36	37.6	36.7		
HB	ND	12.1	14.3	ND	ND		
PAR.D/ml	0	0	0	1973.33	0		
#CELLS	100,000	50,000	50,000	50,000	50,000		
3420	T1	T2	T3	T4	T5	T6	
DATE	3.10.95	31.10.95	28.11.95	11.1.96	6.2.96	5.3.96	
PHA-SI	16.34	8.64	2.25	2.81	23.43	7.93	
E-KNG-SI	2.18	2	0.53	0.69	0.47	1.2	
EXO-N-SI	3.65	3.36	2.03	0.85	-----	-----	
TEMP	36.6	36.5	35.1	35.9	36.4	36.2	
HB	11.6	9.4	17.9	ND	ND	ND	
PAR.D/ml	0	8346.67	0	0	1866.67	746.67	
#CELLS	100,000	50,000	100,000	50,000	100,000	100,000	
3306	T1	T2	T3	T4			
DATE	5.10.95	31.10.95	30.11.95	7.3.96			
PHA-SI	18.68	4.46	6.62	9.18			
E-KNG-SI	2.38	0.41	0.8	1.32			
EXO-N-SI	38.93	1.05	0.69	-----			
TEMP	36.5	36.6	35.6				
HB	9.2	8	10.4	ND			
PAR.D/ml	6826.67	7440	0	826.67			
#CELLS	100,000	100,000	100,000	100,000			

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

3006	T1	T2	T3	T4	T5	T6	T7
DATE	3.10.95	18.10.95	1.11.95	28.11.95	16.1.96	6.2.96	7.3.96
PHA-SI	49.5	9.74	0.44	4.09	11.44	7.24	3.82
E-KNG-SI	2.34	0.38	0.14	0.54	6.74	0.63	0.78
EXO-N-SI	4.09	16.01	2.6	1.1	-----	-----	-----
TEMP	36.6	37.8	37.3	36.6	38.6	36.8	36.9
HB	9.5	ND	10.1	16	ND	ND	ND
PAR.D/ml	0	0	0	0	31120	0	0
#CELLS	50,000	50,000	40,000	50,000	30,000	100,000	50,000
2967	T1	T2	T3	T4	T5		
DATE	2.11.95	16.11.95	14.12.95	18.1.96	8.2.96		
PHA-SI	3.53	42.1	415.96	29.52	1.61		
E-KNG-SI	1.11	1.17	2	0.83	0.49		
EXO-N-SI	0.83	0.57	19.82	-----	-----		
TEMP	36.8	38.2	36.7	36.3	35.9		
HB	10.8	8.9	6.1	ND	ND		
PAR.D/ml	0	21120	3200	0	0		
#CELLS	100,000	50,000	50,000	50,000	100,000		
3356	T1	T2	T3	T4	T5		
DATE	3.10.95	2.11.95	7.12.95	11.1.96	6.2.96		
PHA-SI	17.08	0.54	9.81	1.34	0.46		
E-KNG-SI	1.2	0.7	1.22	0.9	0.41		
EXO-N-SI	0.48	0.24	1.03	0.58	-----		
TEMP	36.2	36.7	39.2	39.2	36.7		
HB	14.4	12.1	12.9	ND	ND		
PAR.D/ml	0	2453.33	21093.33	5146.67	1360		
#CELLS	100,000	50,000	100,000	100,000	100,000		
3369	T1	T2	T3	T4	T5	T6	T7
DATE	3.10.95	31.10.95	30.11.95	11.1.96	8.2.96	20.2.96	7.3.96
PHA-SI	0.1	3.77	5.5	7.91	4.76	7.47	8.61
E-KNG-SI	0.67	0.91	0.87	1.64	0.73	2.71	0.95
EXO-N-SI	0.22	23.45	0.42	1.28	-----	-----	-----
TEMP	36.7	36.3	36.9	36.6	35.7	-----	36.6
HB	6.7	7.6	13.3	ND	ND	ND	ND
PAR.D/ml	8480	1200	0	0	4133.33	0	160
#CELLS	40,000	100,000	100,000	50,000	100,000	100,000	100,000
3435	T1	T2	T3	T4	T5	T6	
DATE	3.10.95	31.10.95	28.11.95	11.1.96	8.2.96	5.3.96	
PHA-SI	3.84	11.17	1.71	13.25	15.92	8.13	
E-KNG-SI	4.51	1.25	1.1	1.42	0.77	0.58	

A SUMMARY OF HISTORY AND PROLIFERATION RESULTS

EXO-N-SI	3.93	1.84	1.52	0.79	-----	-----	
TEMP	37.1	37	36.2	36.5	38	36.5	
HB	11.6	11.1	15	ND	ND	ND	
PAR.D/ml	0	0	0	0	0	0	
#CELLS	26,000	100,000	100,000	50,000	50,000	100,000	
3265	T1	T2	T3	T4	T5		
DATE	31.10.95	28.11.95	11.1.96	8.2.96	5.3.96		
PHA-SI	1.23	25.85	7.74	51.36	16.42		
E-KNG-SI	0.52	6.07	0.78	1.15	1		
EXO-N-SI	0.6	2.43	0.78	-----	-----		
TEMP	37	36.2	36.7	37.8	36.2		
HB	9.5	16.3	ND	ND	ND		
PAR.D/ml	0	0	0	0	0		
#CELLS	100,000	50,000	50,000	50,000	50,000		
3508	T1	T2	T3	T4	T5	T6	
DATE	15.11.95	30.11.95	12.12.95	10.1.96	31.1.96	6.3.96	
PHA-SI	14.2	18.9	0.83	54.93	24.42	11.07	
E-KNG-SI	0.7	0.56	0.71	0.9	1.82	0.83	
EXO-N-SI	0.41	0.32	0.75	0.59	-----	-----	
TEMP	36.5	37.5	37.3	36.1	36.8		
HB	14.9	16.8	18.8	13.3	ND	ND	
PAR.D/ml	0	106.67	0	186.67	0	0	
#CELLS	100,000	50,000	50,000	100,000	50,000	50,000	
3730	T1	T2	T3	T4			
DATE	15.11.95	18.1.96	13.2.96	5.3.96			
PHA-SI	22.68	38.92	45.4	1.02			
E-KNG-SI	1.02	0.17	1.17	1.9			
EXO-N-SI	0.38	-----	-----	-----			
TEMP	36.3	37	37.8				
HB	11.1	ND	ND	ND			
PAR.D/ml	0	27093.33	23093.33				
#CELLS	50,000	40,000	50,000	50,000			
3052	T1	T2	T3				
DATE	2.11.95	9.1.96	6.2.96				
PHA-SI	8.98	9.01	2.46				
E-KNG-SI	0.41	0.73	0.6				
EXO-N-SI	0.96	1.53	-----				
TEMP	36.6	37	36.7				
HB	8.4	13	ND				
PAR.D/ml	6826.67	4293.33	0				
#CELLS	100,000	50,000	100,000				

A SUMMARY OF SUPERNATANT ELISA (Cytokine concentrations in pg/100 ul of sup.)

				CYTOKINE RESPONSES					
				(PHA)		E-KNG		EXO-N	
STUDY#	DATE	PAR.D	TEMP.	IFN	IL4	IFN	IL4	IFN	IL4
3385	19.09.95	1946.67	36.2	722	0	0	0	0	0
3385	19.10.95	0	36.3	309	0	0	0	6	0
3385	22.11.95			1960	0	190	0	70	0
3036	17.10.95	1600	36.6	418	187	0	0	0	353
3036	20.11.95			1280	0	200	0	450	0
3036	20.12.95			320	0	0	0	0	0
2970	23.11.95			1040	0	700	0	550	0
2970	19.10.95	0	38.1	345	0	25	0	25	0
2970	20.12.95			250	0	0	0	480	0
3480	19.09.95	453.33	36.4	579	0	152	157	0	412
3480	19.10.95	2586.67	36.5	407	494	0	374	33	324
3404	19.09.95	0	36.4	1057	291	0	111	0	352
3404	19.10.95	0	36.8	257	0	8	0	0	0
3404	20.11.95			930	0	350	0	510	0
3034	19.09.95	3600	36.4	469	486	0	1357	0	1225
3034	17.10.95	4400	36.9	0	227	0	124	0	0
3001	29.11.95			1100	0	10	0	310	0
3001	26.09.95	4186.67	36	331	0	0	47	0	0
2934	19.09.95	0	37.1	858	0	0	0	0	0
2934	20.11.95			1030	0	300	0	580	0
2927	05.10.95	0	36.1	500	0	552	0	0	0
2927	09.10.95	133.33	39.4	1451	0	0	0	7	0
2927	20.11.95			390	0	110	0	490	0
2927	20.12.95			20	0	0	0	0	0
3421	19.09.95	5840	36.9	671	0	0	0	0	110
3421	05.10.95	1013.33	39.3	1115	0	0	0	0	0
3421	17.10.95	0	36.7	457	28	0	44	0	75
3421	20.11.95			680	0	160	0	430	0
3219	19.09.95	506.67	36.7	151	0	0	0	0	0
3219	17.10.95	2480	36.4	0	0	0	0	0	97
3219	20.11.95			1100	0	140	0	480	0
3219	20.12.95			590	0	0	0	0	0
3091	19.09.95	1146.67	36.5	1089	33	0	168	0	22
3091	17.10.95	0	36.4	457	0	0	0	0	503
3091	20.11.95			1260	0	30	0	550	0
3091	20.12.95			530	0	0	0	0	0
2862	12.10.95	533.33	36.8	0	117	0	441	0	3239
2936	12.10.95	0	36.4	38	0	0	0	0	176
3476	12.10.95	1120	36.9	1485	0	199	0	97	0
3463	12.10.95	0	36.5	0	220	0	287	0	587
3463	07.11.95	0	35.7	518	23	0	36	0	37
3019	07.11.95	0	37.6	0	1406	0	346	0	400
3019	13.10.95	0	36.8	187	621	0	313	0	0
3019	11.12.95			0	0	0	0	0	0
3342	12.10.95	0	36.9	0	12	0	0	0	30
3342	07.11.95	2213.33	37.5	278	0	34	0	0	0
3342	11.12.95			380	0	0	0	0	0
3399	09.11.95	10373.33	37	204	0	0	0	0	231
3399	04.12.95			800	0	0	0	0	0
3027	12.10.95	0	36.6	82	428	0	370	0	515
3027	09.11.95	0	36.7	371	0	0	0	0	0
3027	13.12.95			50	0	0	0	0	0
3401	12.10.95	53.33	37.6	486	0	15	0	125	38
3401	09.11.95	0	36.6	354	9	0	0	0	412

A SUMMARY OF SUPERNATANT ELISA (Cytokine concentrations in pg/100 ul of sup.)

3401	13.12.95			780	0	0	0	0	0
3401	16.01.96			1600	0	0	0	90	0
3429	25.10.95	0	38.5	504	633	0	0	0	0
3429	09.11.95	0	36.8	627	0	0	0	0	0
2955	26.10.95	7733.33	36	335	0	49	0	0	243
2955	27.11.95			1280	0	380	0	580	0
3100	26.09.95	4186.67	36.6	873	0	0	0	0	0
3100	24.10.95	0	36.3	105	0	0	0	0	246
3100	27.11.95			1590	0	270	0	190	0
3344	26.09.95	19013.33	36.3	192	0	0	0	0	0
3344	24.10.95	800	36	254	0	33	0	23	0
3344	29.11.95			680	0	0	0	40	0
2998	24.10.95	7093.33	36.6	0	31	0	33	0	700
3359	24.10.95	0	36.9	0	0	0	0	0	0
3440	19.10.95	986.67	36.3	435	0	0	0	0	0
3440	20.11.95			1880	0	340	0	600	0
3484	21.09.95	53.33	36.7	335	0	0	0	0	0
3484	22.11.95			780	0	0	0	350	0
3484	27.11.95			190	0	280	0	160	0
2819	21.09.95	3093.33	36	726	0	0	0	61	331
2819	19.10.95	0	36	218	52	0	506	50	0
2819	22.11.95			670	0	100	0	140	0
2819	18.12.95			1700	0	1300	0	320	0
2901	21.09.95	906.67	36.4	186	46	0	0	111	0
2901	19.10.95	453.33	36.9	84	246	0	320	0	313
2901	22.11.95			1690	0	30	0	190	0
2835	05.10.95	11920	36	483	0	0	0	0	428
2835	31.10.95	2746.67	36	73	0	0	0	92	0
2859	03.10.95	0	36.2	0	0	0	0	0	0
2974	05.10.95	1840	35.7	651	0	0	0	0	0
3304	05.10.95	0	36.5	645	0	0	152	0	1982
3304	02.11.95	0	36	308	0	0	0	23	0
3304	04.12.95			0	0	0	0	40	0
3420	03.10.95	0	36.6	0	248	0	0	0	0
3420	12.10.95			24	0	0	0	30	0
3420	31.10.95	8346.67	36.5	124	271	0	427	0	758
3420	04.12.95			0	0	0	0	0	0
3306	31.10.95	7440	36.6	525	68	0	3	0	95
3306	05.10.95	6826.67	36.5	215	0	0	0	0	0
3306	06.11.95			280	0	0	0	0	0
3006	03.10.95	0	36.6	0	0	0	0	0	0
3006	18.10.95	0	37.8	42	165	0	0	0	407
3006	01.11.95	0	37.3	0	68	0	127	2	334
3006	04.12.95			620	0	220	0	280	0
2967	02.11.95	0	36.8	492	31	0	0	10	158
2967	22.11.95			1380	0	370	0	400	0
2967	20.12.95			1590	0	1600	0	0	0
3356	03.10.95	0	36.2	823	301	0	61	0	214
3356	02.11.95	2453.33	36.7	94	121	0	114	0	0
3356	13.12.95			200	0	0	0	0	0
3356	17.01.96			210	0	0	0	0	0
3369	03.10.95	8480	36.7	0	0	0	250	0	561
3369	31.10.95	1200	36.3	211	32	6	142	0	148
3369	06.11.95			0	0	0	0	0	0
3369	17.01.96			10	0	0	0	0	0
3435	03.10.95	0	37.1	0	0	0	0	0	0
3435	31.10.95	0	37	187	234	0	340	48	282
3435	04.12.95			0	0	0	0	160	0

A SUMMARY OF SUPERNATANT ELISA (Cytokine concentrations in pg/100 ul of sup.)

3435	17.01.96			220	0	0	0	0	0
3265	31.10.95	0	37	77	157	49	0	22	267
3265	04.12.95			0	0	0	0	0	0
3265	17.01.96			0	0	0	0	0	0
3508	21.11.95			360	0	10	0	270	0
3508	06.11.95			480	0	0	0	0	0
3508	18.12.95			0	0	0	0	0	0
3508	16.01.96			430	0	0	0	0	0
3770	21.11.95			580	0	380	0	30	0
3770	22.11.95			700	0	110	0	1150	0
3770	18.12.95			0	0	0	0	0	0
3761	21.11.95			520	0	140	0	50	0
3761	20.12.95			1640	0	380	0	1530	0
2513	22.11.95			1110	0	100	0	210	0
2513	18.12.95			410	0	0	0	0	0
2821	22.11.95			1620	0	30	0	290	0
2821	18.12.95			1100	0	0	0	210	0
3254	22.11.95			410	0	300	0	140	0
3397	22.11.95			1710	0	180	0	710	0
3397	20.12.95			0	0	0	0	0	0
3411	22.11.95			1050	0	160	0	640	0
3148	23.11.95			1130	0	490	0	800	0
3148	20.12.95			0	0	0	0	0	0
3148	16.01.96			70	0	0	0	0	0
3142	13.12.95			1390	0	0	0	0	0
3524	18.01.96			0	0	0	0	0	0
3524	23.11.95			10	0	0	0	390	0
3199	23.11.95			0	0	0	0	280	0
3052	15.01.96			0	0	0	0	0	0

TABLE 1: A SUMMARY OF PBMC PROLIFERATIVE RESPONSES TO PHA FROM STUDY SUBJECTS BETWEEN SEPTEMBER 1995 TO MARCH 1996

SAMPLE	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.
3385	19.97	5.47	6.79	13.05	45.03	ND	ND
3524	ND	ND	7.75	ND	22.52	4.29	12.25
3036	ND	7.24	9.85	120.31	4.86	12.7	ND
2970	ND	2.44	5.05	13.59	3.14	23.52	ND
3404	31.24	4.26	18.31	ND	32.34	4.15	ND
3034	45.78	28.43	ND	ND	13.65	9.11	ND
3001	3.88	ND	12.35	ND	21.79	2.72	ND
2821	ND	ND	101.64	23.64	33.79	4.47	ND
2934	ND	ND	19.77	12.04	ND	4.87	ND
2927	ND	40.45	37.56	74.45	45.75	12.43	ND
3421	23.18	0.42	16.65	7.2	45.75	ND	ND
3219	29.02	17.58	34.09	14.67	18.939.55	4.02	ND
3091	ND	11.76	8.81	53.22	32.28	5.66	ND
3411	ND	ND	2.72	ND	10.68	10.42	2.57
3254	ND	ND	2.3	ND	5.81	25.9	7.35
3199	ND	ND	21.01	ND	24.02	6.09	8.82
2936	ND	9.32	ND	11.21	6.42	62.43	ND
3148	ND	ND	5.05	45.9	0.59	93.73	10.26
3463	ND	13.29	99.39	ND	18.93	42.96	8.67
3397	ND	ND	68.17	16.58	7.03	10.09	ND
3019	ND	22.68	14.36	5.47	26.01	ND	ND
3342	ND	3.3	1.81	18.59	16.39	3.05	ND
3399	ND	8.88	52.24	19.87	7.07	5.05	ND
3142	ND	ND	4.83	23.68	0.36	61.27	ND
3027	ND	7.79	18.61	6.86	15.37	2.39	ND
3401	29.73	15.24	35.63	2.45	2.36	7.48	6.5
3429	7.01	25.49	39.03	ND	ND	15.84	ND
3100	4.71	2.7	38.3	ND	47.32	34.76	ND
3344	27.13	11.79	30.85	58.43	15.43	ND	ND
3761	ND	ND23.3	28.11	ND	18.15	45.11	ND
3770	ND	ND	5.01	31.32	7.61	31.3	ND
2513	ND	ND	38.6	62.44	2.33	41.6	ND
3440	ND	63.13	6.82	ND	6.92	7.91	0.55
3484	27.71	ND	4.23	18.17	16.71	9.19	15.21
2819	58.61	8.46	27.63	243.33	1.95	0.45	ND
2901	100	27.04	6.64	ND	31.68	ND	ND
2835	ND	14.37	5.83	ND	ND	7.93	8.16
3304	ND	2.72	0.51	15.55	1.28	ND	9.98
3420	16.34	8.64	2.25	ND	2.81	23.43	7.93
3306	18.68	4.46	6.62	ND	ND	ND	9.18
3006	49.5	9.74	0.44	4.09	11.44	7.24	3.82
2967	ND	3.53	42.1	415.96	29.52	1.61	ND
3356	17.08	0.54	9.81	ND	1.34	0.46	ND
3369	0.1	3.77	5.5	7.91	4.76	7.47	8.61
3435	3.84	11.17	1.71	ND	13.25	15.92	8.13
3265	ND	1.23	25.85	ND	7.74	51.36	16.42
3508	ND	14.2	18.9	0.83	54.93	24.42	11.07

ND STANDS FOR NOT DONE

TABLE 1: A SUMMARY OF PBMC PROLIFERATIVE RESPONSES TO PHA FROM STUDY SUBJECTS BETWEEN SEPTEMBER 1995 TO MARCH 1996

3730	ND	ND	22.68	ND	38.92	45.4	1.02
3052	ND	ND	8.98	ND	9.01	2.46	ND
	ND STANDS FOR NOT DONE						

ND STANDS FOR NOT DONE

TABLE 2: A SUMMARY OF THELYMPHOPROLIFERATIVE RESPONSES OF INFANTS TO MSP-1 19 kDa BETWEEN SEPTEMBER 1995 TO FEBRUARY 1996 (S.I. VALUES)

			FOLLOW UP MONTHS			
SAMPLE	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.
3385	0.38	0.76	1.17	0.88	ND	ND
3524	ND	ND	1.29	ND	2.05	0.71
3036	ND	0.48	1.23	1.38	0.42	0.61
2970	ND	0.46	0.43	0.89	0.73	2.6
3404	1.15	0.58	1.61	ND	1.12	0.54
3034	1.61	0.96	ND	ND	1.01	5.94
3001	0.96	ND	1.43	ND	1.84	2.67
2821	ND	ND	2.81	0.79	0.83	0.22
2927	ND	2.89	2.31	5.68	0.94	0.64
3421	2.31	0.26	0.58	0.94	ND	ND
3219	1.94	4.23	2.73	2.2	0.81	1.02
3091	ND	0.76	1.02	1.34	1.03	0.69
3411	ND	ND	0.6	ND	0.72	1.57
3254	ND	ND	0.42	ND	0.76	0.81
3199	ND	ND	0.79	ND	0.27	0.58
3397	ND	ND	3.5	0.79	0.72	0.58
2936	ND	2.53	ND	4.12	0.7	0.94
3148	ND	ND	0.43	2.8	0.77	1.56
3463	ND	0.71	0.86	0.54	1.08	2.05
3019	ND	0.95	0.83	1.63	ND	0.7
3342	ND	2.84	0.78	0.86	0.49	0.65
3399	ND	1.18	1.17	1.8	0.72	1.49
3142	ND	ND	ND	0.88	1.88	1.05
3027	ND	0.44	1	2.63	1.51	0.86
3401	2.15	0.77	1.65	0.7	1.13	0.39
3429	ND	0.3	0.51	1.85	ND	0.78
3100	1.84	0.57	1.41	ND	0.51	1
3344	0.28	0.89	0.93	ND	2.64	2.45
3761	ND	ND	0.78	0.53	ND	0.72
3770	ND	ND	0.79	1.12	1.22	0.95
2513	ND	ND	1.05	1.84	1.21	0.77
3440	ND	1.07	2.01	ND	0.9	0.7
3484	2.62	ND	0.81	0.64	1.27	1.02
2819	0	0.42	1.31	0.99	0.98	0.99
2901	0.6	0.67	0.26	ND	3.63	ND
2835	ND	1.05	0.64	ND	ND	2.48
3304	ND	1	0.63	1.21	1.82	ND
3420	ND	2.18	2	0.53	0.69	0.47
3306	ND	2.38	0.41	0.8	ND	ND
3006	2.34	0.38	0.14	0.54	6.74	0.63

ND STANDS FOR NOT DONE

TABLE 2: A SUMMARY OF THELYMPHOPROLIFERATIVE RESPONSES OF INFANTS TO MSP-1 19 kDa BETWEEN SEPTEMBER 1995 TO FEBRUARY 1996 (S.I. VALUES)

2967	ND	1.11	1.17	2	0.83	0.49
3356	ND	1.2	0.7	1.22	0.9	0.41
3369	0.67	0.91	0.87	1.64	0.73	2.71
3435	4.51	1.25	1.1	ND	1.42	0.77
3265	ND	0.52	6.07	ND	0.78	1.15
3508	ND	0.7	0.56	0.71	0.9	1.82
3730	ND	ND	1.02	ND	0.17	1.17
3052	ND	ND	0.41	ND	0.73	0.6

ND STANDS FOR NOT DONE

TABLE 3: A SUMMARY OF LYMPHOPROLIFERATIVE RESPONSES OF PBMC FROM INFANTS TO EXO ANTIGENS BETWEEN SEPTEMBER 1995 TO JANUARY 1996 (S.I. Values)

		FOLLOW UP MONTHS			
SAMPLE	SEPT.	OCT.	NOV.	DEC.	JAN.
3385	0.35	7.06	0.54	0.38	ND
3036	ND	0.63	1.52	3.95	0.49
2970	ND	3.78	0.67	1.71	ND
3404	0.54	7.34	0.85	ND	ND
3034	0.99	21.66	ND	ND	0.7
2927	1.59	1.58	0.76	5.38	ND
3421	0.63	19.46	1.43	0.56	0.7
3219	0.68	2.05	2	0.37	1.16
3091	ND	0.62	0.36	1.52	ND
3148	ND	ND	0.29	2.03	0.56
3019	ND	22.24	2.26	1.39	ND
3342	ND	1.6	0.35	0.93	ND
3399	ND	ND	1.15	1.03	1.59
3027	ND	0.84	1.84	0.41	ND
3401	ND	1.61	0.85	1.96	1.24
3429	ND	0.86	8.85	2.32	ND
3100	8.73	1.56	1.21	ND	ND
3344	13.74	0.96	1.19	ND	ND
3484	0.84	ND	0.52	1.28	ND
2819	1.21	4.67	0.58	1.17	ND
2901	0.52	6.03	0.22	ND	ND
3304	ND	1.03	0.76	11.62	0.59
3420	ND	3.65	3.36	2.03	0.85
3306	ND	38.93	1.05	0.69	ND
3006	4.09	16.01	2.6	1.1	ND
2967	ND	ND	0.83	0.57	19.82
3356	ND	0.48	0.24	1.03	0.58
3369	ND	0.22	23.45	0.42	1.28
3435	ND	3.93	1.84	1.52	0.79
3265	ND	0.6	2.43	0.78	ND
3508	ND	ND	0.41	0.32	0.75

ND STANDS FOR NOT DONE

TABLE 4: A SUMMARY OF CYTOKINE RESPONSES TO PHA, MSP-1 19kDa AND EXO ANTIGENS

			IL-4				IFN		
DATE	SAMPLE	MED	PHA	E-KNG	EXO-N	MED	PHA	E-KNG	EXO-N
19.09.95	3421	542	0	0	110	0	671	0	0
19.09.95	3404	335	291	111	352	0	1057	0	0
19.09.95	3480	496	0	157	0	20	579	152	0
19.09.95	3385	966	0	0	0	0	722	0	0
19.09.95	3034	519	486	1357	1225	0	469	0	0
19.09.95	3091	1240	33	168	22	0	1089	0	0
19.09.95	3219	948	0	0	0	0	151	0	0
19.09.95	2934	877	0	0	0	0	858	0	0
21.09.95	2901	1029	46	0	0	0	186	0	111
21.09.95	3484	0	0	0	0	0	335	0	0
21.09.95	2819	0	0	0	331	0	726	0	61
26.09.95	3001	1367	0	47	0	0	331	0	0
26.09.95	3100	441	0	0	0	0	873	0	0
26.09.95	3344	0	0	0	0	0	192	0	0
28.09.95	2026	0	41	0	388	0	0	0	0
28.09.95	3122	219	0	0	0	125	53	0	0
28.09.95	2065	0	0	0	0	0	1185	0	0
28.09.95	3513	0	0	0	0	0	317	0	0
28.09.95	3492	552	0	0	0	0	0	0	0
28.09.95	3371	336	0	8	0	0	902	0	0
03.10.95	3369	815	0	250	561	0	0	0	0
03.10.95	3420	878	248	0	0	0	0	0	0
03.10.95	3435	810	0	0	0	0	0	0	0
03.10.95	3356	368	301	61	214	0	823	0	0
03.10.95	3006	1198	0	0	0	0	0	0	0
03.10.95	2859	517	0	0	0	0	0	0	0
05.10.95	2835	353	0	1.10E+04	428	0	483	0	0
05.10.95	3421	0	0	0	0	0	1115	0	0
05.10.95	2974	0	0	0	0	0	651	0	0
05.10.95	3306	1083	0	0	0	0	215	0	0
05.10.95	3304	0	0	152	1982	327	645	0	0
05.10.95	2927	0	0	0	0	0	500	552	0
09.10.95	2927	1746	0	0	0	0	1451	0	7
12.10.95	3476	0	0	0	0	109	1485	199	97
12.10.95	3420	0	0	0	0	0	24	0	30
12.10.95	2862	0	117	441	3239	0	0	0	0
12.10.95	3463	0	220	287	587	267	0	0	0
12.10.95	2936	155	0	0	176	0	38	0	0
12.10.95	3401	237	0	0	38	0	486	15	125
12.10.95	3027	40	428	370	515	80	82	0	0
12.10.95	3342	367	12	0	30	0	0	0	0
13.10.95	3019	398	621	313	0	327	187	0	0
17.10.95	3091	337	0	0	503	0	522	0	0
17.10.95	3421	375	28	44	75	0	457	0	0
17.10.95	3036	299	187	0	353	0	418	0	0
17.10.95	3034	415	237	124	0	5.7	0	0	0
17.10.95	3219	380	0	0	97	0	0	0	0
18.10.95	3006	859	165	0	407	0	42	0	0
19.10.95	2970	599	0	0	150	0	345	25	61
19.10.95	3404	920	0	0	0	4	257	8	0
19.10.95	3480	369	494	374	324	0	407	0	33
19.10.95	2819	525	52	509	0	0	218	0	50
19.10.95	3385	903	0	0	0	0	309	0	6
19.10.95	2901	393	249	320	313	0	84	0	0
19.10.95	3440	1400	0	0	0	0	435	0	0
17.10.95	2998	35	31	33	700	0	0	0	0
17.10.95	3100	342	0	0	246	0	105	0	0
17.10.95	3344	386	0	0	0	0	254	33	23
17.10.95	3359	1141	0	0	0	0	0	0	0
25.10.95	3429	385	633	0	0	0	504	0	0

TABLE 4: A SUMMARY OF CYTOKINE RESPONSES TO PHA, MSP-1 19kDa AND EXO ANTIGENS

26.10.95	2955	429	0	0	243	0	335	49	0
31.10.95	3306	370	68	3	95	0	525	0	0
31.10.95	3420	102	271	427	758	0	124	0	0
31.10.95	2835	1187	0	0	0	0	73	0	92
31.10.95	3369	272	32	142	148	1	211	6	0
31.10.95	3265	243	157	0	267	0	77	49	22
31.10.95	3435	347	243	340	282	0	187	0	48
01.11.95	3006	379	68	127	334	0	0	0	2
02.11.95	2967	470	31	0	158	0	492	0	10
02.11.95	3356	526	121	114	0	260	94	0	0
02.11.95	3304	591	0	0	0	0	308	0	23
02.11.95	3052	574	0	0	52	0	440	0	0
07.11.95	3342	788	0	0	0	0	278	34	0
07.11.95	3463	298	23	36	37	0	518	0	0
07.11.95	3019	294	1406	346	400	0	0	0	0
09.11.95	3401	530	9	0	412	0	354	0	0
09.11.95	3429	690	0	0	0	0	627	0	0
09.11.95	3027	600	0	0	0	0	371	0	0
09.11.95	3399	525	0	0	231	0	204	0	0