Helminthes & Immune Responses to Mycobacterial Antigens

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List of Abbreviations

AHRI- Armauer Hansen Research Institute

AIDS- Acquired immuno deficiency syndrome

ALP- Alkaline Phosphatase

APC- Antigen Presenting Cell

BCG- Bacille Calmette Guerin

CD- Cluster of Differentiation

DEC- Diethyl carbamazine

DMSO- Dimethyl sulphoxide

ELISA- Enzyme Linked Immunosorbant Assay

HIV- Human Immunovirus

IFN-Interferon

IG-Immunoglobulin

IL- Interleukin

KTTC- Kotebe Teacher's Training College

LAK- Lymphokine activated killer cell

MHC- Major Histocompatibility Complex

NK- Natural Killer cell

PBMC- Peripheral Blood Mononuclear Cell

PBS- Phosphate Buffered Saline

PHA- Phytohaemaglutinin

PPD- Purified Protein Derivative

STCF-Short Term Culture Filtrate

TH1- T helper Type 1

TH2- T helper type 2

TU- Tuberculin Unit

WHO - World Health Organization

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Abstract

Tuberculosis has become the major killer disease globally. As it is the case with most other infectious diseases the magnitude of the problem is the highest in resource poor countries. There are several means to control the disease. One of these is the use of vaccine (BCG). However, the vaccine offers the least protection against pulmonary tuberculosis, the most infectious form of the disease, in areas where TB is most prevalent. Part of the explanation for this may be that chronic infectious disease particularly, helminthes, in these areas may polarize the immune response towards humoral type when cell mediated immunity takes the lions share in defending the body against intracellular infections. In order to assess whether intestinal helminthes could influence mycobacteria specific immunity, we investigated the *in vivo* and *in vitro* mycobacteria specific immune responses of helminth exposed population at Kotebe Teacher's Training College. Our finding was that deworming significantly enhanced the *in vitro* lymphocyte proliferative responses, IFN-γ and IL-10 production in response to mycobacterial antigens and a T cell mitogen, PHA, compared to untreated controls. Moreover, the initially PPD non reactive subjects were BCG vaccinated and later tested for mycobacteria specific immune responses *in vivo* and *in vitro*. We found that BCG, when given after deworming caused significant improvement in T cell proliferation and IFN-γ production compared to pre-vaccination levels in response to a mycobacterial antigen, PPD, whereas in the placebo group the difference was not significant. This finding support the notion that intestinal helminthes may impair immune responses against mycobacterial infections and that BCG vaccination may confer better protection if given after deworming in helminth exposed population which is an already established notion in veterinary medicine.

1. Introduction

Tuberculosis is a chronic infectious disease caused in most cases by *Mycobacterium tuberculosis*, an acid-fast rod shaped bacillus and occasionally by *M. bovis*. *M. tuberculosis* is a facultative intracellular parasite which requires aerobic conditions for growth (Fine, 1994). Mycobacteria contain abundant lipids and waxes, which are responsible for the hydrophobicity and intracellular persistence of the bacterium (Kaufmann,1991).

M. tuberculosis, although it can infect any cell, prefers to infect mononuclear phagocytes. Their long life span of months and their limited antibacterial capacity against acid fast bacilli makes resident tissue mononuclear phagocytes ideal candidates for colonization by *M. tuberculosis* (Dannenberg, 1991).

1.1 Immunity in Tuberculosis

Infection occurs when inhaled bacilli reach alveoli of the lung. An alveolar macrophage ingests the bacillus and it often destroys it. But still some replication of the bacilli may occur in the macrophage. As some of the bacilli are chewed up in the phagolysosomes, certain proteins or polypeptides are released from the phagolysosomes and are transported to the surface of the macrophage where they are presented to the T lymphocytes in association with the MHC molecules of the host cell (Fine, 1994). These complexes are then recognized by T cells bearing complementary receptors that trigger the acquired T cell mediated immune response and the emergence of acquired resistance (Orme and McMurray, 1996).

It is still not known what protective epitopes of the baccillus are actually presented nor is it known why this interaction leads to the sensitization of the protective T cells as well as other CD4 $^{+}$ T cell subsets that appear to be functionally different such as those mediating DTH (Tsukada *et al.,* 1991).

When a T lymphocyte recognize the presented antigen, various key immunomodulators (cytokines) are released. Particularly IL-1 by macrophage and IL-2 by T cells. This in turn induce the T cell to proliferate into clones of cells with particular specificities (Fine, 1994). Sensitized T cells, upon interaction with infected macrophage, begin to secrete their own array of cytokines. Many of these influence other T cells and in turn, the T cell derived cytokines influence macrophage activity (Orme and McMurray, 1996).

CD4 $^{+}$ T cells are grouped into two based on their cytokine profile; those that produce the cytokines IL-2 and IFN-γ are termed Th1, while those that secrete IL-4, IL-5, IL-6 are termed Th2. It was demonstrated that the Th1 CD4^{+}T cells play pivotal role in the subsequent pathogenesis and immune response in tuberculosis (Kehrl *et al.*, 1986). The cytokines produced by these cells activate macrophage to efficiently ingest and digest mycobacteria and secrete other cytokines such as IL-6, IL-8 and TNF (Orme *et al.*, 1993).

IFN and TNF synergize to maximize the antimocrobial effects of macrophage (proton pump and production of reactive oxygen metabolites), both of which enhance phagolysosomal fusion and directly damage the bacilli (Chan *et al.*, 1992).

Interaction between macrophage and T cells is essential for the control of *M. tuberculosis* infection. Initially, infected macrophages present mycobacterial antigens in association with MHC class I, II or with non polymorphic MHC like proteins (CD1) (Fenton and Vermeulen, 1996). Macrophage derived cytokines play critical roles in the generation of antigen specific T cells and cytokines produced by activated T cells in turn modulate macrophage function. Th1 type cytokines may enhance antigen presentation and stimulate antimicrobial responses within the infected macrophage itself.

On top of the CD4 dependent processes, there is evidence that the CD8 series are also involved in the response to *M. tuberculosis* infection (Kaufmann, 1988). These cells can be directly cytotoxic to the infected macrophage. This cytotoxicity may also destroy the bacilli or release them into the extracellular environment so that they would be engulfed and killed by other activated macrophages (Fine, 1994).

1.2 Mechanisms of persistence of *M. tuberculosis* in Mononuclear phagocytes

Uptake of bacteria into phagosomes is usually followed by fusion of phagosomes with lysosomes and subsequent degradation of bacteria. It has been indicated that virulent mycobacteria can inhibit phagolysosomal fusion possibly through the effect of surface sulfatides (Goren *et al.*, 1976). These pathogens can also escape from fused phagolysosomes into membrane bound vesicles that then fails to fuse with secondary lysosomes (Britton *et al.*, 1994). It was also shown that

mycobacteria can disrupt phagosome membrane and escape into the cytoplasm where they can manage to replicate (McDonough *et al.*, 1993).

Pathogenic mycobacteria may establish infection within macrophages by resisting their microbicidal activity which is affected by reactive oxygen and nitrogen intermediates, the production of which is stimulated mainly by T cell derived cytokines (mainly IFN-γ and TNF-α) (Kuhn &Goebel, 1994). Cell envelope components, mainly glycolipids interfere at different stages of these processes such as modulating the release of the important cytokines, blocking activation of macrophages by cytokines or scavenging toxic oxygen and/or nitrogen radicals (Britton *et al.*, 1994; Chan *et al.*, 1991; Sherman *et al.*, 1995).

1.1 Impaired Cell mediated immunity Predisposes to Tuberculosis

The strongest risk factor for the development of tuberculosis is coinfection with HIV (Selwyn *et al.*, 1989). HIV infection causes reactivation of latent tuberculosis, increases the risk of primary infection and enhances susceptibility to reinfection (Small *et al.*, 1993). This shows that there is a strong association between impairment of cell mediated immune response in HIV infected individuals and the development of tuberculosis.

In AIDS patients tuberculosis is often characterized by poorly formed granulomas (gross fibrosis surrounding a central area of necrosis meant to wall off infectious foci), inability of macrophage to kill ingested mycobacteria, large bacterial load and hematogenous spread of the disease (Nithingale *et al.*, 1992; Theur *et al.*, 1990). It was noted that tuberculosis in the HIV^+ could occur often

when the $CD4^+$ count is either normal or minimally depressed. This suggests that other defects in the cell mediated immunity contribute to the greater susceptibility (Ravn, 1997). Such defects may include loss of memory $CD4^+$ T cells that occur at an early stage of HIV infection (Helbert *et al.*, 1993), antigen specific CD4+ T cell mediated cytotoxicity and NK/LAK cell activity (Forte *et al.*, 1992).

1.4 Epidemiology of Tuberculosis

For many years the incidence of tuberculosis was declining at least in the developed world and the disease was predicted to be eradicated by the year 2010 (Bloom & Murry, 1992). However, in the year 1989-92, for instance 30% increase in the disease incidence occurred in the U.S.A. (CDC, 1992). Several factors contributed to this reversion of the world tuberculosis problem:

- crowding in refugee camps, prisons
- the emergence of multidrug resistant TB
- host factors causing increased risk of contracting and developing TB as intravenous drug abuse, alcoholism, malnutrition and
- the HIV pandemic

The global magnitude of tuberculosis is enormous. Dolin *et al* (1994) estimated that about 90 million new cases of tuberculosis is expected to occur worldwide through the year 1990-1999. Tuberculosis is the world's principal cause of death among adults from a single infectious agent (Bloom and Murray, 1992). Moreover, Sudre *et al* (1992) estimated that approximately 1.7 billion people, about a third of the world population is infected with *M. tuberculosis.* The great majority of these reside in the developing world.

1.4.1 Future Global Incidence

Without the recognition of tuberculosis crisis confronting the world and prompt effective action, the tuberculosis epidemic can be expected to worsen for several reasons:

- demographic forces - children born in the past decades in regions with high population growth rate are now reaching the ages at which morbidity and mortality of tuberculosis is high
- famine, war, and natural disasters which create large population of displaced, malnourished people in crowded living condition
- HIV/AIDS will be of increasing importance in tuberculosis epidemiology.

Projections made in 1989 indicate that the incidence of tuberculosis can be expected to increase to 10.2 million cases by the year 2000, and 11.9 million by the year 2005 (Di Perri *et al.*, 1989). Assuming that the availability and effectiveness of treatment programs remain at the 1990 level, 3.5 million deaths can be expected to occur by the year 2000 (Dolin *et al.*, 1994). While only 4.6% of tuberculosis deaths were attributable to HIV infection in 1990, the proportion of deaths attributable to HIV will increase to more than 14% by the year 2000 (Daley *et al.*, 1992).

The HIV epidemic may also worsen the problem of drug resistance in tuberculosis. This is because HIV infected persons who subsequently become infected with *M. tuberculosis* have a very high risk of developing active

tuberculosis within a short period. Thus HIV can increase tuberculosis epidemic of both resistant and susceptible strains, with its manifestation occurring in months rather than years (Daley *et al.,* 1992).

1.5 Immunity in Helminth Infection

Gastro- intestinal worms are estimated to infect about 1 billion people worldwide and believed to cause 1 million deaths a year. Children in developing countries are particularly prone to infection. There are about 20 species of helminthes that are natural parasites of man. Others cause zoonotic diseases. In Africa, each individual will harbor, on average, two species of parasite (Muller and Morera, 1994).

Unlike microparasites (viruses, bacteria, or protozoa), helminthes don't multiply within the human body (Tedla, 1986). As a result, helminth diseases do not have sudden acute crisis but tend to be chronic afflictions where disease severity is proportional to worm burden which is often affected by the intensity of transmission in the area (Chan, 1997).

In humans the most striking features of helminthes include:

- long term persistence within the host
- the ability to elicit protective immunity only after many years or decades of exposures
- complex developmental cycles (Maizels *et al.*, 1993).

The persistent nature of infection with progressive accumulation of parasites, in addition to their impact on the immune system can induce chronic sequelae such as hepatosplenic diseases induced by *Schistosoma mansoni,* lymphoedema and elephantiasis induced by lymphatic filariasis and blindness caused by *Onchcerca vulvulus*. Long term infection, if associated with poor nutrition can lead to impaired physical and cognitive development in children with high worm loads (Muller & Morera, 1994). In addition to their direct effect on the body, gastrointestinal worm infections could predispose to secondary bacterial or other parasitic infections (Christensen *et al.*, 1987).

In an endemic area, an infected individual may harbor the parasitic worms for most of her/his life owing to repeated exposure to infection and inability to develop effective protective immunity (Maizels *et al.*, 1993). Such infectious background may also alter the host's ability to cope with subsequent infections (Christensen *et al.*,1987).

Immunological responses to parasitic helminth infections are characterized by three distinct elements:

- elevated IgE antibody production
- eosinopilia and
- mastocytosis- each of these responses appear to be controlled by cytokines from the Th2 subset of $CD4^+$ T cells: IgE by IL-4 (Finkelman *et al.*, 1988) eosinophilia by IL-5 (Limaye *et al.*, 1990), mastocytosis by IL-3, IL-4 and IL-10 (Mahanty *et al.*, 1992).

Furthermore, human T lymphocyte clones or populations with characteristic Th2 cytokine production profile can be derived from parasitized hosts by stimulation with worm antigens or a mitogen (Sher and Coffman, 1992). Much of the current work on helminth immunology is focused on the question of whether this Th2 response plays a functional role in immunity and how it is selectively induced. Some information can be drawn from epidemilogical correlation, but the understanding so far rests heavily upon animal model systems and in vitro studies (Finkelman *et al.*, 1991).

The prime candidates for effective immune mechanisms against helminth organisms are the humoral and cellular components that show preponderance during infection. The most distinctive of these features is a dramatic elevation of serum IgE levels in humans (Capron and Dessaint, 1985). The nematode *Nippostrongylus brasiliensis* raise total the serum IgE levels (Ogilvie *et al.*, 1966). The potentiation of IgE is mediated by soluble mediator, IL-4 (Finkelman *et al.*, 1990), the major source of which is Th2 $CD4^+$ T cells.

Eosinophilia is another hallmark of helminth infections. It is known that in human helminthiasis more than 50% of circulating white blood cells may be eosinophil granulocytes. This is in contrast to their normal levels of 2-5%. Similarly, in rodent infections, circulating eosinophil numbers increase 10-30 fold, a phenomenon blocked by antibody against IL-5. (Coffman *et al.*, 1989).Eosinophils are thought to be effector cells in antibody mediated cellular cytotoxicity.

Infections with helminths also provoke mastocytosis, particularly in the intestinal mucosa. This is mediated by IL-3, IL-4 and IL-10 (Mohanty *et al.*, 1992). Which of these responses that preferentially or in concert, protects the host against helminth parasites is yet to be resolved. In human schistosomiasis, both elevated eosinophilia and specific IgE responses correlate with resistance to re-infection after chemotherapy (Dunne *et al.*, 1992), whereas in filariasis, patent microfilaraemia is associated with depressed IgE response (Kurniawan *et al.*, 1993). However, IgE mediated responses are linked with pathology rather than resistance in human trichuriasis infections (Cooper *et al.*, 1991).

In vitro model show that helminth parasites can be killed by IgE mediated mechanisms involving platelets, macrophages and eosinophils (Capron and Dessaint, 1985). Eosinphils kill larval schistosomula in vitro by deposition of toxic granule proteins in synergy with reactive oxygen intermediates (Yazdanbakhsh *et al.*, 1987) and hypoeosinophilic animals have impaired resistance against migrating larvae of *Strongloides venezulensis* and *Trichinella spiralis* (Grove *et al.*, 1977).

Taken together, the description above indicate that chronic helminth infection would polarize the immune response towards a Th2 type (the arm of the immunity that plays the major role in the defense against extracellular pathogens) as can be seen from increased IgE, eosinophilia and mastocytocysis which are all typical of Th2 immunity. Such polarized environment is not without an impact on cell mediated immunity, which is important in the defense against intracellular pathogens such as HIV, *M. tuberculosis*. This has been demonstrated by several

investigators (Bassily *et al.*, 1987; Bentwich *et al.*, 1996; Anzala *et al.*, 1996; Greene *et al.*, 1985; Ghaffar *et al.*, 1989).

1.6 Mucosal Immunity

The mucous membranes covering the epithelial linings of the gastrointestinal tract, respiratory tract etc are supplied with specialized immune system to protect itself and the body's interior against potential dangers. It is responsible for the production of the mucosal immune response which is distinct form the systemic immune response(Czerkinsky & Holmgren, 1995). It functions under a common "mucosal immune system" and involves more than 80% of the total Ig producing cells (Brandtzaeg et al., 1989). Its normal function is to modulate the mucosal immunity by active down regulation, immune exclusion through secretion of IgA and IgM and suppression of active immunity mainly to dietary antigens but also inocous antigens from pathogenic and non-pathogenic organisms. Some times these mechanisms fail to adequately exclude the antigen and an active inflammatory reaction takes place.

In the gut the absorption of antigens in sufficient quantities by epithelial cells covering peyers patch will be processed and presented to the underlying B and T lymphocytes. A portion of these antigen sensitized B and T cells then leave their site of stimulation and migrate through lymphatics to the circulatory system and populate areas remote from their stimulation sites. These arising from GALT, for example can discriminate to various secretory tissues including the gut, mammary and salivary glands and respiratory tracts (Quiding *et al.*, 1996).

In contrast the mucosal epithelial cells from lack of co-stimulatory molecules could produce clonal deletion, clonal anergy and or induction of suppressor cells

what is known as oral tolerance. When it occurs it produces unwanted and potentially deleterious effect on the host systemic immunity. That is the nonspecific inhibition of the systemic immunity. This is one mechanism mucosal pathogens like parasites that deliver their immunogen enterically weaken the immunity of their hosts. The crucial variable as to which mechanism (clonal deletion, clonal anergy or bystander cell suppression) is trigered seem to be dose of antigen which corresponds to intensity of infection.

As opposed to the sysytemic immune apparatus that functions in a normally sterile milieu, the MALT functions in an environment full of inocous antigens. This shows that the MALT must function in such a way to avoid a bystander tissue damage and immunological exhaustion. The MALT is populated by B cell, T cell and accessory cell subpopulations. Antigens taken up by absorptive epithelial cells and specialized epithelial cells called membrane cells (M cells) can be channeled to APC or presented directly to the underlying B and T cells by epithelial cells (Quiding-Jarbrink *et al.*, 1996).

The immune response with the majority of antigens results in suppression of specific immunity and in some cases active immunity develops. The balance between active immunity /suppression depends on the nature of antigen, the type of accessory cells, lymphocytes involved and host genetics (Quiding-Jabrink *et al.*, 1995).

Active immune responses here are of two major types:

- antibody formation which involves mainly secretary IgA
- cellular immunity (Mestecky & McGhee, 1987)

1.7 The differentiation of T helper subsets

Differentiated T helper cells are grouped into two subsets Th1 and Th2 characterized by distinct cytokine profiles (Mosmann *et al.*, 1986). Such distinction (Th1/Th2 pattern) does not exist among naïve T cells or perhaps even among long term memory cells. When first stimulated, naïve $CD4^+$ T cells principally produce IL-2, and then differentiate into phenotypes that secrete other cytokines. Thus, Th1 and Th2 cells can be derived from a single precursor cell (Mosmann and Sad, 1996).

Differentiation of uncommitted T cell precursors is influenced by cytokines. IL-4 stimulates differentiation into Th2 cells, whereas IFN-γ, IL-2 and TGF-beta enhance Th1 development (Cherwinski *et al.*, 1987). IFNγ promotes Th1 development in two ways: by enhancing IL-12 secretion by macrophage and by maintaining the expression of IL-12 receptors on CD4+ T cells, rendering the cells more responsive to IL-12 (Guler *et al.*, 1996). It has minor Th1 inducing effect independent of IL-12 (Abbas *et al.*, 1996). These cytokines are provided early in a primary response by cells other than the antigen specific T cells (Mosmann and Sad, 1996). IL-12 is produced by several cell types particularly macrophages in response to certain microbial products. NK cells produce IFN-γ in response to IL-12 or antigen mediated cross-linking of antibody bound to FC receptors.

In mice, early IL-4 may be contributed by mast cells, basophils and sub-populations of $CD3^+CD4^+$ $NK1.1^+$ T cells that appear to be restricted by MHC

class I molecules (Yashimoto and Paul, 1994). These cells have a restricted T cell receptor repertoire, unknown antigen specificity and produce large amounts of IL-4 few hours after polyclonal stimulation (Mosmann and Sad, 1996).

Different antigen presenting cells (APCs) may exert selective influences on T cell differentiation depending on the cytokines they secrete. Further more, co-stimulatory molecules on APCs (B7.1 and B7.2) may selectively influence T cell differentiation (Janeway and Traverse, 1996).

How the immune system makes the important decision of whether Th1 or Th2 cells develop is not clear but it is expected that multiple and precise mechanisms in the immune system translate the properties of the pathogen in to cytokine or co-stimulatory signals that induce the appropriate type of Th cells and effector functions.

In vivo experiments have implicated antigen dose as an important factor in the Th1/Th2 decision. Low antigen doses were found to be associated with Th1 responses whereas high doses favor Th2 responses (Bretcher *et al.*, 1992; Hosken *et al.*, 1995; Power *et al.*, 1998). However, the consequences of antigen dose may depend on the soluble or particulate nature of antigen. At low concentration of soluble antigenic peptide, each APC should present a low density of peptide MHC complexes. On the other hand, if the antigen is a particulate one, although relatively few APCs capture the antigen, each successful APC present substantial amounts because of the digestion of the whole particle. Such situation can be exaggerated if the pathogen is localized to small foci of infection. Thus, low dose

may mean a low amount of antigen on all APCs, or higher dose on few APCs. Such situation may deserve a separate due consideration (Mosmann and Sad, 1996).

Not few authors also argue that the choice of Th1/Th2 is dependent on genetic influences. A good example of genetic control of Th1/Th2 development is the resistance /or susceptibility of inbred mice to infection with *Leishmania major*, which correlates with specific Th1 or Th2 responses respectively. *Ex vivo* studies indicate that $CD4^+$ T cells from mice resistant or susceptible to *L. major* infection differ in their intrinsic tendency to develop towards Th1/or Th2 in response to a protein antigen other than *L. major* (Hsieh *et al.*, 1995). T cells from susceptible mice acquire a more Th2 like phenotype and lose IL-12 responsiveness more rapidly than CD4 + T cells from resistant phenotype. This difference is attributable to greater initial IL-4 production by cells from susceptible mice and more prolonged maintenance of IL-12 responsiveness in cells from the resistant phenotype (Guler *et al.*, 1996). The molecular basis of such effect remains to be defined.

1.8 Control of Tuberculosis

Control measures against tuberculosis are intended either to protect individuals or to break the cycle of infection. This can be achieved through improvements in socioeconomic conditions, case finding and treatment, vaccination, and chemoprophylaxis (Rodrigues and Smith, 1990).

Socioeconomic development has the most profound effect in reducing the disease load, as tuberculosis is highly associated with poverty. Case finding and treatment is a strategy for short-term control of tuberculosis and is aimed at reducing the risk of transmission. Chemoprophylaxis is treatment given to healthy infected persons to prevent the progression from infection to disease and it makes smear positive cases non-infectious (WHO, 1994).

The other means employed to control tuberculosis is the use of vaccination. The vaccine Baccilus Calmette Guerin (BCG) is the oldest, the most widely used, and is the only one available against tuberculosis. Nevertheless, it is also the most controversial vaccine with highly variable protective efficacy against pulmonary tuberculosis from nil to approximately 80% (Fine, 1994). However, in some countries like Sweden the general BCG vaccination of the newborn was abondoned since 1975 because of the epidemiological situation and also due to the relatively large number of BCG related complications. Today only those belonging to risk groups are vaccinated (Ohman, 1995). This variability as most authors argue could be due to among others varying exposure to environmental mycobacteria (Palmer and Long, 1966; Cheng *et al.*, 1993) other co-existing diseases and source and type of vaccine. An overview of published literature in 1994 by Colditz and co-workers shows that environmental mycobacterial exposure could explain only about 41% of the observed variability.

Another explanation for BCG's inconsistent behavior is genetic or physiological differences among the trial populations. In the mouse, immune response to mycobacterial infection is under genetic control (Skamene, 1989). One of the best

studied genes affecting resistance/or susceptibility to infections with mycobacteria is the BCG gene in the mouse (Gros *et al.*, 1981). However, there is no evidence in human to support a genetic explanation for BCG's inconsistent behavior in man. In fact an epidemiological study have shown that BCG protects Asians living in Britain much better than those living in Asia (Packe & Innes, 1988). More over, there is no evidence so far that the efficacy of BCG varies with nutritional status.

The other possible factor for variability in BCG's efficacy may be immune perturbation by chronic infectious diseases. Most areas where the vaccine offers the least protection are characterized by high endemic prevalence of chronic infectious diseases, particularly helminths (Hori *et al.*, 1993). The host exposed to infectious burden would be expected to respond to the challenge, this would alter the normal immune balance (Bentwich *et al.*, 1996) and consequently, the host immune response to subsequent infections.

Moreover, in veterinary medicine there is a common belief that in order to obtain maximal protective effects of vaccines against intracellular pathogens in domestic animals, there is a need for deworming the animals first (Sven Britton pers.comm.). There is now a rationale based on the modern concepts of dichotomy of the immune response behind this procedure. This study was aimed at investigating the possible effects of intestinal helminth infections on the *in vivo* and *in vitro* immune response to mycobacterial antigens before and after BCG vaccination.

Objective(s):

General:

- To determine the effect of deworming on existing and/or induced immunity to *Mycobacterium tuberculosis* complex.

Specific:

- To determine the effect of deworming on the *in vivo* and *in vitro* immune response to mycobacterial antigens

- To investigate the effect of deworming on BCG induced immunity compared to untreated controls.

2. Materials and Methods

2.1 Study Population

The study was carried out on volunteer students at the Kotebe Teacher's Training College in Addis Ababa, Ethiopia. The objectives and the details of the study were presented and those willing to participate were registered. The study population consisted of mainly male students with age ranging from 18-24 (mean 21.6 years) and employees of the college age range 26-50 (mean 36.5 years). Those who were pregnant, known cases of HIV, and TB were excluded. The population was randomly grouped into two (albendazole and placebo). Ethical clearance from the local medical and national medical ethical committee was obtained and informed verbal consent was also obtained from the study subjects. The college administration as well as the student's association agreed to the study.

2.2 Parasitological examination

Stool samples were collected in a cap and transported to AHRI at room temperature. The samples were examined using direct microscopy and the Formol-Ether concentration techniques.

2.2.1 Direct Microscopy

Using a wooden applicator, about 2mg of stool was transferred onto a glass slide and a drop of 0.9% sodium chloride solution was added onto the sample on a glass slide and mixed until a thin smooth suspension was made. The preparation was then covered with cover slip and the entire preparation was then examined under the microscope.

2.2.2 The Formol-Ether stool parasite concentration method

An estimated 2 grams of stool was mixed with 10% formalin solution. The stool formalin mixture was passed through a strainer to remove any large particles, then 3 ml of ether was added and the suspension was mixed and centrifuged. Parasite cysts, eggs ans larvae were sedimented and the stool debris was separated. The supernatant with debris was discarded and the sediment transferred onto glass slide for microscopic examination.

2.3 Treatment

400mg single dose of albendazole was administered twice a month apart to the experimental group and the control group remained untreated until the test was done, rather were provided with placebo (tablets containing all components of albendazole except the pharmacologically active one) following the same protocol. The BCG vaccinated subjects were kept on albendazole/or placebo at a dose of 400mg/month throughout the study period. At the end of the study, the placebo group were treated with albendazole. No apparent side effect is associated with the dose employed.

2.4 Tuberculin Skin Testing

Two units of purified protein derivative (PPD-RT23) with tween 80 obtained from Statens Serum Institut, Copenhagen, Denmark, was used for the test. The test was done by intradermal administration of 0.1ml (2TU) tuberculin in the back of the left mid-forearm using disposable syringes and needles. The diameter of skin reaction was measured 72 hours later using the ball point technique of Sokal (1975) where a line is drawn with moderate pressure with a ball point pen from a point 1-2 cm away from the margin of skin test reaction towards it center. When the ball point reaches the

margin of the indurated area, definite resistance to further movement is noted; the pen is then lifted. This procedure was repeated from the opposite side of the reaction. The lines drawn by the pen provide a visible record of the margins of induration and the distance between the opposing lines can be measured accurately by a caliper meter.

2.5 Antigens

Phytohaemaglutinin (PHA) obtained from Wellcome Diagnostics, Dartford, UK (3ug/ml). Purified Protein Derivative (PPD) at a concentration of 4 ug/ml from Statens Serum Institut, Denmark, and secreted proteins from a 7 day old culture of virulent *M. tuberculosis* termed short term culture filtrate (ST-CF) (gift from Dr. Pernille Ravn) were used for the *in vitro* assays.

2.6 In vitro immunological works

2.6.1 Isolation of Peripheral Blood Mononuclear Cells (PBMC)

At the time of PPD setting 10ml of venous blood was obtained from the study population into heparinized vaccutainer tube and transported to AHRI laboratory at room temperature. The blood samples were diluted 1:1with RPMI-1640 and layered on Ficoll-paque (Pharmacia, Sweden) at a Ficoll to blood ratio of 1:3. This was centrifuged at 2000rpm for 25 minutes at room temperature. The layer of PBMC was collected into 15ml polystyrene tube and washed three times with RPMI-1640 at room temperature. Cell pellet was resuspended in 10% dimethylsulphoxide (DMSO) with fetal calf serum, aliquoted into nunc tubes and step-wise frozen to $-70^{\circ}C$ and preserved under liquid nitrogen until assay.

2.6.2 In Vitro Stimulation

Cryopreserved PBMCs were thawed in a water bath at 37°C and washed three times in RPMI-1640 to remove the DMSO. PBMC pellet was resuspended in complete RPMI-1640 containing 1% penicillin, 1% streptomycin, 1% glutamine, 10% heat inactivated normal human AB serum. Viability and number was determined by trypan blue staining. Over 90% of cells were found to be viable at the time of assay.

2.6.3 Proliferation assay

The cell suspension was adjusted to $1x10^6$ cells/ml and seeded at 200ul/well in a round-bottomed microtiter plate. The cultures were set in triplicate and cells were cultured without antigen (negative control), with PHA at a concentration of 3ug/ml, PPD at 5ug/ml and ST-CF at 1ug/ml. The cultures were maintained in a humidified incubator with 5% CO_2 at 37°C. 100ul of culture supernatants were collected at 72 hours from PHA stimulated cultures and at day 5 from PPD and STCF stimulated cultures. Culture supernatants were stored in a freezer at –70°C until assay.

20 hours before the termination of the culture (at day 3 for PHA stimulated culture and day 5 for mycobacterial antigen stimulated cultures) 10ul of saline containing 1uci ^{3}H-thymidine (TRA120, Amersham Radiochemical Center, UK) was added to the cultures. Pulsed cultures were collected on a filter mat (Cat.No.11731) using cell harvester (Skatran, Norway) and dried for 1-2 hours in an oven. ^{3}H-thymidine incorporation was measured in a scintillation fluid by beta-scintillation counter (1216 Rackbetta II, Finland).

2.6.4 Cytokine Analysis:

2.6.4.1 IFN-γ Assay

IFN-γ levels were determined using sandwich ELISA. In brief, wells of Dynatech Immunol plates were coated overnight at 4°C with 2ug/ml monoclonal antibody

(MoAB 1-D1K). After washing three times with Saline +Tween (0.05%), plates were incubated with phosphate buffered saline (PBS) plus tween at room temperature for 1 hour. Samples diluted 1:2 and standards (100ul) were added to the wells and further incubated at room temperature for 1.5 hours. Biotinylated secondary monoclonal antibody at 1ugg/ml (7-B6-1 Biotin) was added after further washing and incubated at room temperature for one hour. Streptavidin -ALP diluted 1:1000 in PBS+Tween was added to the wells after another washing step and incubated for 1 hour at room temperature. This was followed by washing and p-nitrophenyl phosphate tablets (Sigma, USA) dissolved in 20ml ddH_2O was added (100ul) and incubated at room temperature for color development reactions. Absorbancy was read at 405nm by an ELISA reader (Titertek Multiskan plus, Finland). As standard reference commercially available recombinant IFN-γ (Gibco Laboratories) was used. 100 pg/ml IFN-γ was determined as the lowest level for positive responses and the results were given as the means of duplicate wells.

2.6.4.2 IL-4 Assay

IL-4 assay employs the standard ELISA procedure using ELISA kit from Boeringer Mannheim, Germany. In brief, monoclonal antibody specific for IL-4 has been precoated onto a microplate. 100ul of standards and 1:2 diluted samples were pipetted into wells and incubated at room temperature for 2hours. After washing a way any unbound substances using wash buffer peroxidase enzyme linked polyclonal antibody specific for IL-4 (anti-h-IL-4-POD) was added to wells (100ul) this was incubated at room temperature for 1hour. This was followed by additional washing step to remove any unbound antibody-enzyme reagent. A substrate solution (tetramethylbenzimidine in stabilized hydrogen peroxide) was added and incubated at room temperature for additional 30 minutes followed by optical density reading at 450nm by an ELISA

reader (Titertek multiskan Plus, Finland). The lowest detection limit of the assay was 6pg/ml.

2.6.4.3 IL-10 Assay

IL-10 assay employs the quantitative sandwich enzyme immunoassay technique. In brief, monoclonal antibody specific for IL-10 has been precoated onto a microplate. 200ul standards and 1:10 diluted samples were pipetted into wells of microtiter plate and incubated at room temperature for 2hours. After washing away any unbound substances using wash buffer (PBS+0.05%Tween) peroxidase enzyme linked polyclonal antibody specific for IL-10 (anti-h-IL-10-POD) was added to wells (200ul) and incubated at room temperature for 1h. This was followed by additional washing step to remove any unbound antibody enzyme reagent. A substrate solution (tetramethylbenzidine in hydrogen peroxide)was added to each well (200ul) and incubated at room termperature for 30 minutes. Finally, 50ul of stop solution(2N sulfuric acid) was added and optical density was read at 450nm in an ELISA reader (Titertek Multiskan Plus, Finland).The kit was obtained from Quantikine TM, R & D systems, UK. The lowest detection limit of the assay was 2 pg/ml.

2.7 Data Analysis

Data were analyzed using Sigma Plot, Sigma Stat, and Excel computer programs and differences in proliferation responses and cytokine production were compared by student's *t-tests*. Data are expressed as means and standard error of the mean. Differences were considered significant when the p-value was less than or equal to 0.05.

3. Results

3.1 Parasitological Examination

The prevalence of intestinal parasites in the study population is summarized in Table 1. The prevalence of parasites was unexpectedly low, 26.7% for worms and 16% for protozoal parasites. The most prevalent helminthic infestation was *Ascaris lumbricoides* (13%), followed by Hook worms (9.2%), *Trichuris trichuria (4.1%)*, *Strongloides stercoralis* (2.3%), *Hymenolepis nana*(2.3%), and *Taenia spp*(1%). *Entamoeba histolytica* (12.3%) and *Giardia lamblia* (7%) were the two common protozoan parasites observed. Overall, intestinal parasites were identified in 40.2% of the subjects. Of these, 26.7% harbor one or more helminthes whereas 18% were positive for protozoal parasites (*Entamoeba histolytica* and *Giardia lamblia*). Double infection with protozoa and helminth occurred in 4.7% of the population.

Among the staff population, prevalence of worm infestation was 8%, and that of protozoa infection was 44%.

An interesting result here was that among the student population helminth infection rate was higher compared to the staff (26.7% *v* 8%) and infection rate by protozoa was higher in the staff population (44% *v*18%).

After albendazole treatment, stool samples were 94% negative for helminthes and the drug had no significant effect on protozoal infection.

Table 1. Prevalence of different intestinal parasites in the study populations in percentage (%).

Parasite	Student (n=211)	Staff(n=50)
Ascaris lumbricoides	13	6.0
Hook worm	9.2	0.0
Trichuris trichuria	4.1	2.0
Hymenolepis nana	2.3	0.0
Strongloides stercoralis	2.3	0.0
Taenia spp.	1	0.0
Entamoeba histolytica	12.5	42.0
Giardia lamblia	*7.0*	10.0
At least one worm	26.7	8.0
At least one protozoa	18	44.0
A protozoa and worm	4.7	6.0

3.2 Tuberculin Skin Test

A tuberculin skin test was done 6 weeks after albendazole or placebo treatment for both groups. The tuberculin positivity rate was higher in the albendazole treated group (69.2%) compared to the placebo group (59.3%). Moreover, the size of the DTH response was also higher in the dewormed group. However, the difference was not significant statistically. (Fig.1&2).

3.3 Skin Test Conversion in the BCG vaccinated

Those persons who were considered Mantoux test negative (<7mm induration to 2 TU) were BCG vaccinated and 1.5 months and 4.5 months later tuberculin skin tests were again performed. In 20 vaccinees tuberculin skin tested 1.5 months after vaccination, all but one became positive (all initially Mantoux negative) irrespective of the treatment (placebo or albendazole), confirming previous findings that BCG vaccination does cause skin test conversion (Das *et al.*, 1998). However, only 5 of the 20 vaccinees (25%) tested 4.5 months after vaccination were found to be PPD positive (Fig.3). This suggests that vaccine induced tuberculin reactivity rapidly wanes with time.

3.4. Lymphocyte Proliferation Assays

T cell proliferative responses to PHA and two mycobacterial antigens, were determined for 29 subjects in each group (Albendazole and Placebo). All subjects had similar *in vivo* responses to tuberculin PPD. Proliferative response to PHA and PPD was significantly higher in the dewormed group compared to their untreated counterparts with p=0.003 & 0.005, respectively (Fig.4). Proliferative responses to ST-CF were also higher in the dewormed group compared to the undewormed, but this was, however, not statistically significant (p=0.06). Helminth free, PPD^+ subjects were included as controls to see if albendazole by itself has any effect on the *in vitro* immune response, to rule out the possibility of the direct immune potentiating effect of the drug. The results (Fig.5) indicate that the drug by itself has no direct effect on the immune response and that the differences in

immunological parameters observed between albendazole and placebo treated populations were due to its effect on the worms.

3.5 T cell proliferation in the vaccinees

PPD negative subjects in both groups were BCG vaccinated and their *in vitro* immune responses to mycobacterial antigens were compared.

T cell proliferative responses were determined for 10 vaccinees before and after BCG vaccination. The dewormed group had significantly improved PPD induced T cell responses, compared to pre vaccination levels (Fig.6). Interestingly, the difference in proliferative response pre and post vaccination in the placebo group was not significant. This may imply that worm infection has an effect on the immunizing potential of BCG. In other words, BCG when delivered to wormfree individuals, may confer better immunity than in the worm-infested ones. But a more comprehensive study is required to conclude that deworming enhances the vaccine efficacy.

3.6 Cytokine Secretion

In order to investigate quantitative differences between the two groups with regard to the cytokines produced by cultured PBMC. Cytokine detection by ELISA was used to quantify the production of IFN-γ, and IL-10. Significant differences were observed with regard to the amount of IFN-γ and IL-10. The dewormed group produced significantly greater amounts of IFN-γ and IL-10 upon *in vitro* stimulation of PBMC with PHA and PPD (Fig.7&9).

The high level of IFN-γ in the dewormed group and the comparatively low level in the control could mean that persons with chronic helminth infection could have weakened immunity against intracellular infections such as *Mycobacterium tuberculosis*.

In addition, elimination of helminth parasite from the body could improve Th1 type immunity as can be seen from the increased IFN-γ production in the dewormed group upon *in vitro* stimulation with PHA and mycobacterial antigens (PPD & ST-CF).

IFN-γ assay in the vaccinees: Albendazole and placebo groups were compared with regard to the change in the level of IFN-γ secretion after BCG vaccination with reference to pre vaccination levels. Of interest here was that the dewormed group produced significantly greater amounts of IFN-γ after vaccination *in vitro* compared to pre-vaccination levels, whereas in the placebo group the difference was not significant (Fig.8).

3.7. IFN-γ v IL-10

Statistically significant positive correlation exists between IFN-γ and IL-10 (Fig.10), cytokines with antagonistic effects i.e. those persons with raised IFN-γ also showed raised levels of IL-10. This may show some degree of feedback interaction between the two cytokines.

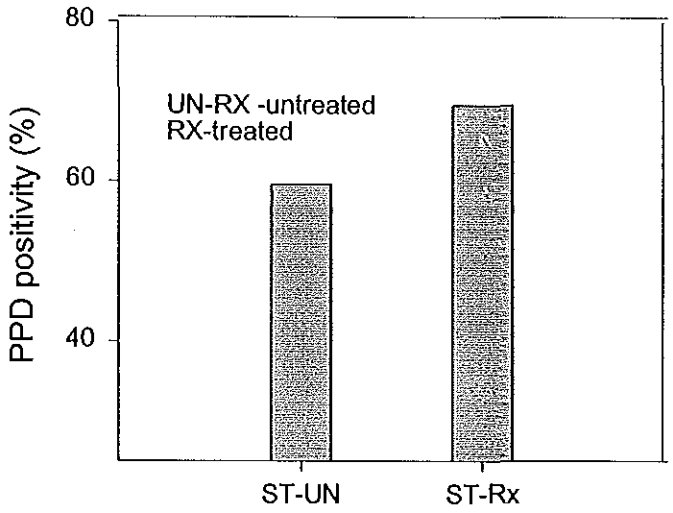


Fig. 1 Prevalence of PPD reactivity in the treated (RX) and untreated (UN-RX) student population.The difference between the two groups is not significant statistically. (St.un, n=109 &ST-Rx, n=79)

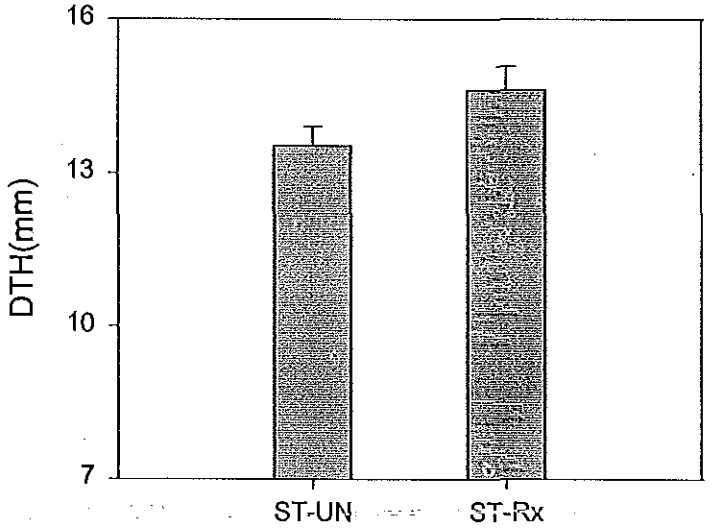


FIG. 2 Difference in the size of DTH reaction (mm) between the treated (ST-RX) and untreated population (ST-UN). The difference is not statistically significant. (DT-UN, n=109, & St-Rx, n=79).

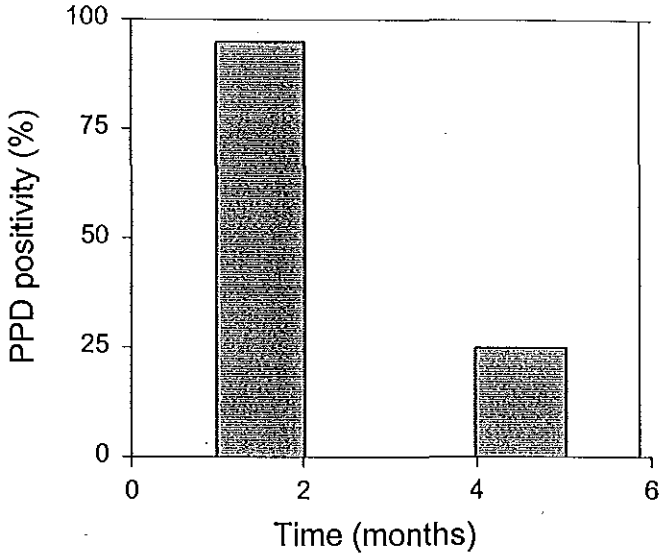


Fig.3 PPD reactivity rate at different time points after BCG vaccination. This shows that BCG induced skin test response rapidly vaines with time (n=20).

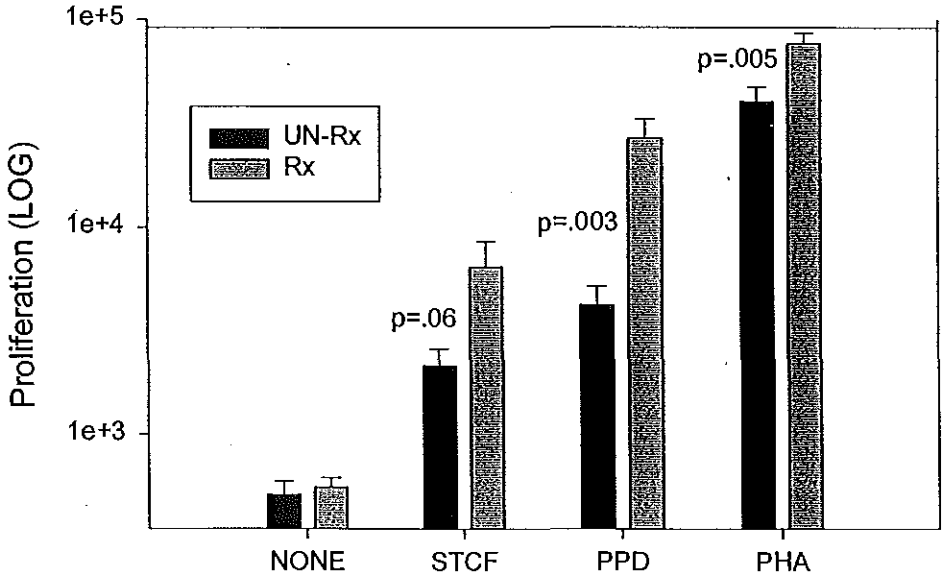


Fig.4Proliferative response of PBMC from untreated (UN-Rx) & treated (RX) population.The difference between the two groups is statistically significant (n=29).

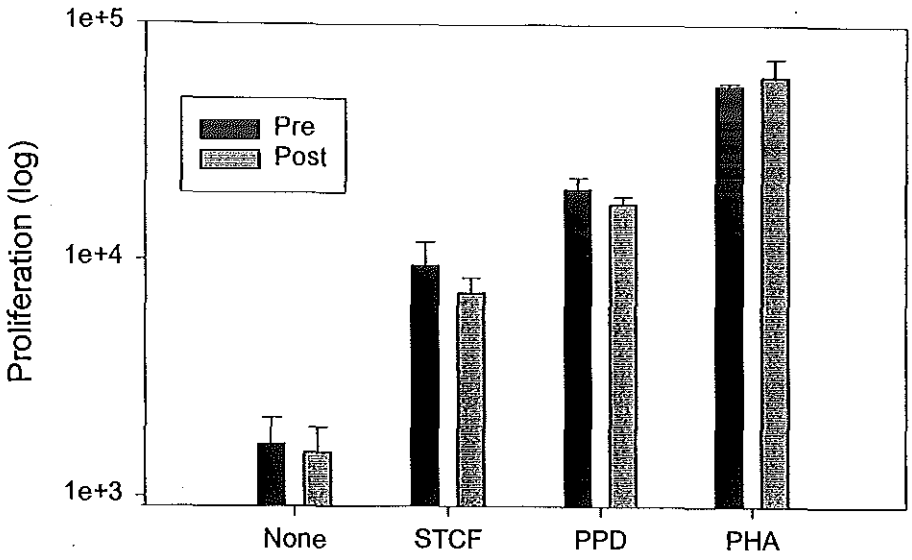


Fig.5 Proliferative response to a mitogen and mycobacterial antigens (PPD&STCF) of PBMC obtained from helminth free individuals pre and post albendazole treatment (n=10). No significant difference in all cases.

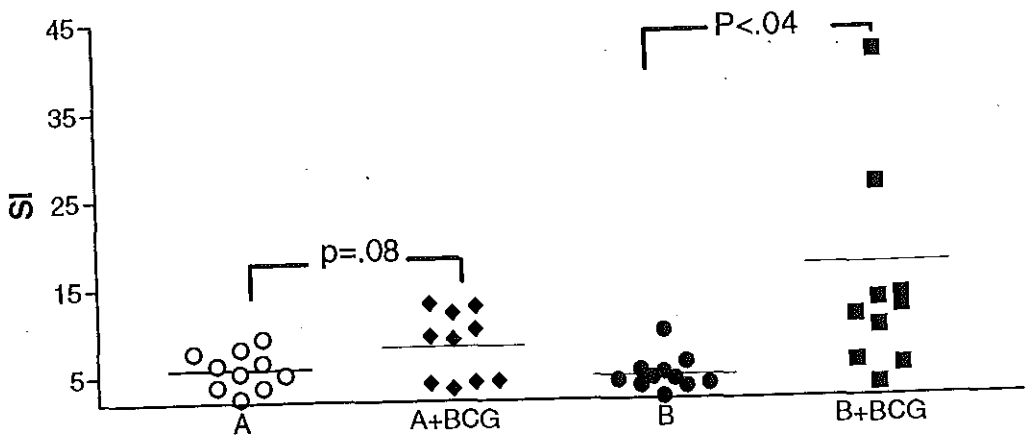


Fig.6 Proliferative response to PPD of PBMC from untreated Pre and post vaccination(A and A+BCG), treated pre and post vaccination (B and B+BCG).

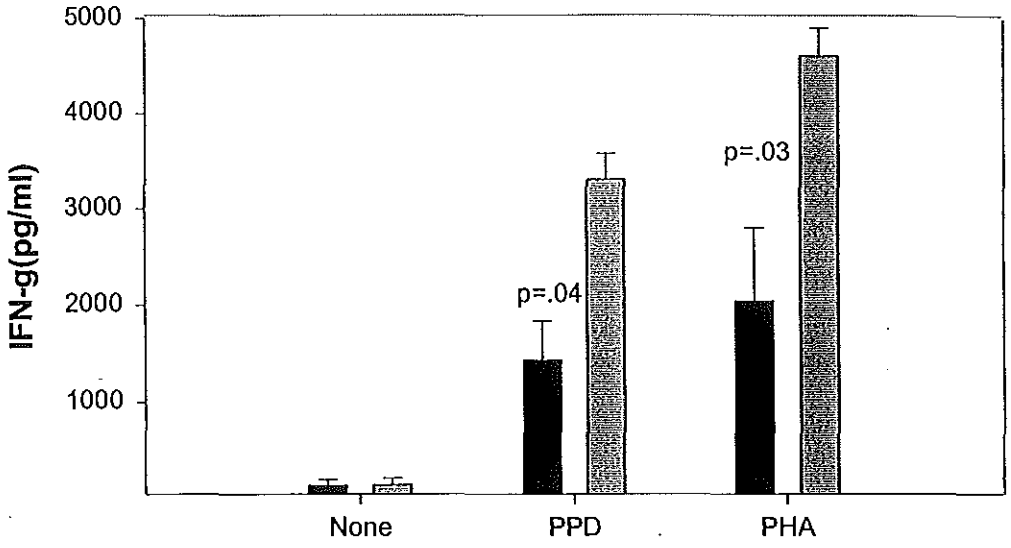


FIG.7 Level of IFN-g in supernatants collected from cultured PBMC obtained from untreated (UN-RX) and treated (RX) population. (n=15).

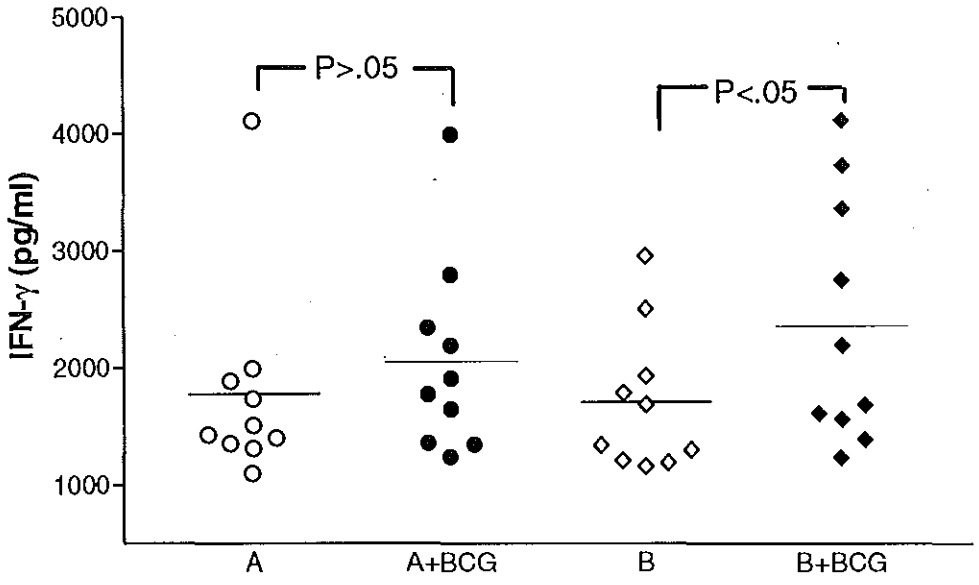


Fig.8 IFN-γ production by PBMC from untreated-unvaccinated (A), untreated-vaccinated (A+BCG), treated-unvaccinated (B) and treated -vaccinated (B+BCG) stimulated in vitro with PPD.

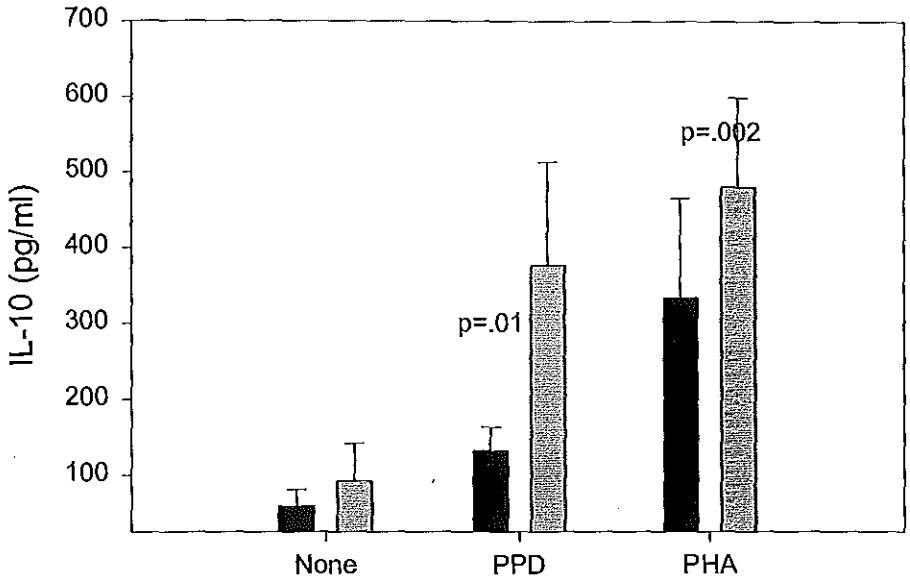


Fig.9 IL-10 secreted from cultured PBMC obtained from treated (RX) and untreated (UN-RX) population.The difference between the two groups is significant (n=15).

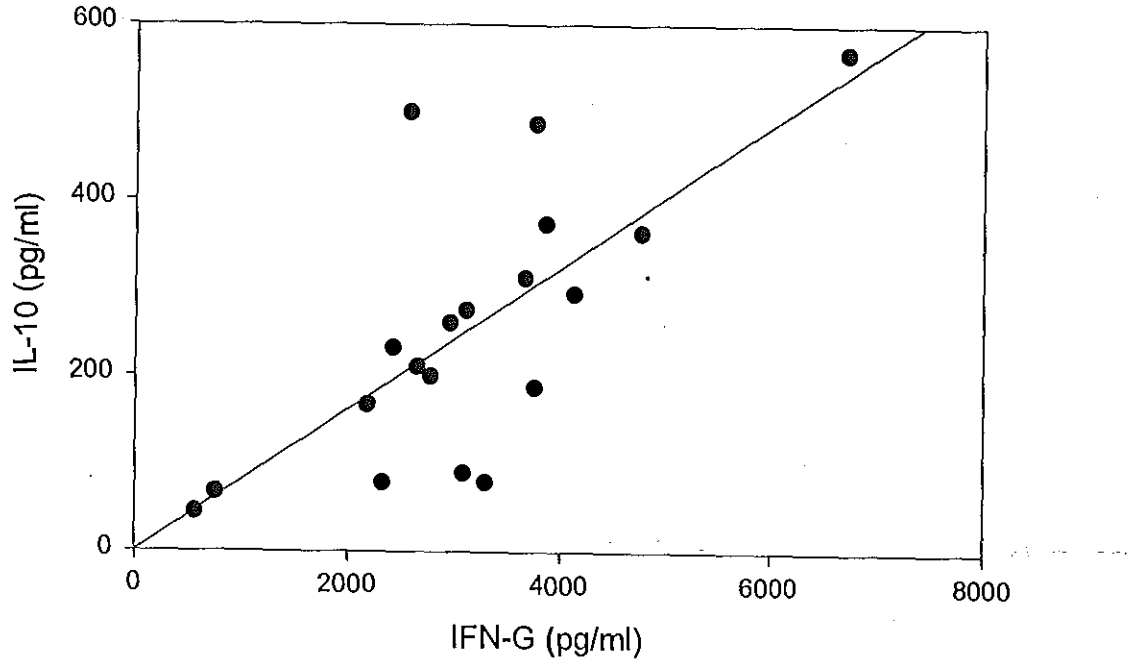
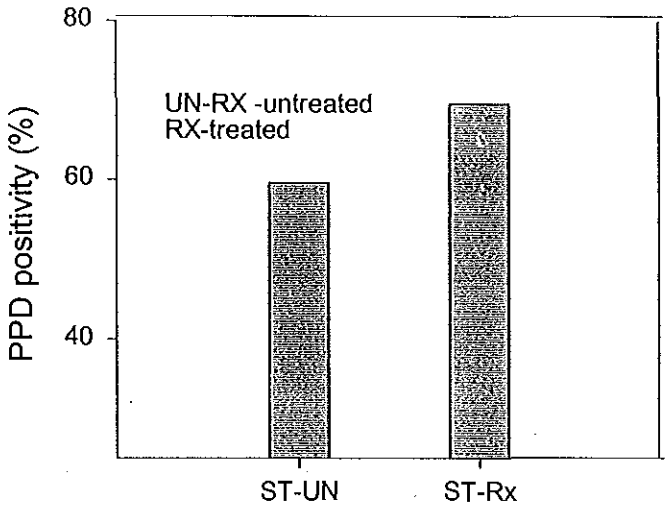


Fig-10 Relationship between IFN-g and IL10. There is a good positive correlation between the two cytokines (r=0.607) N=19.



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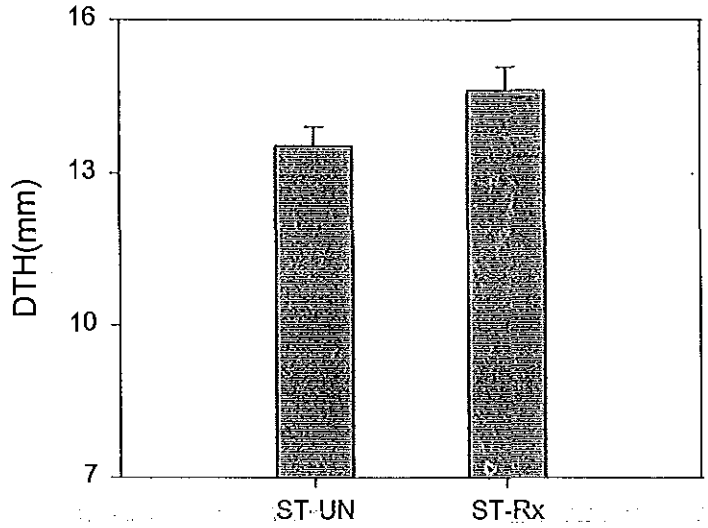

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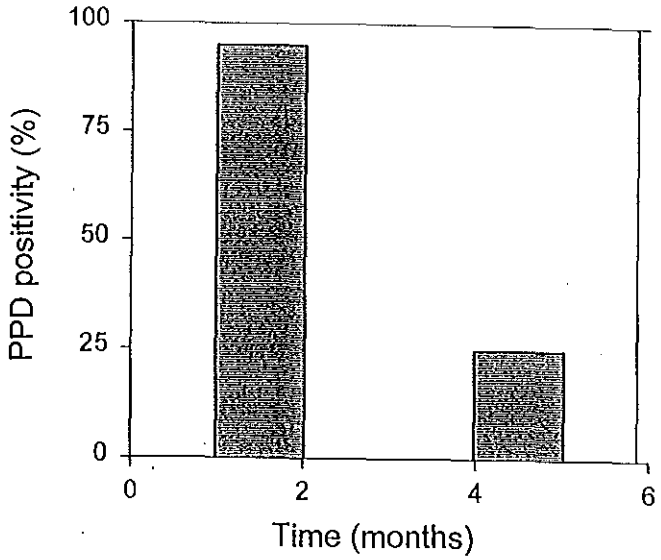

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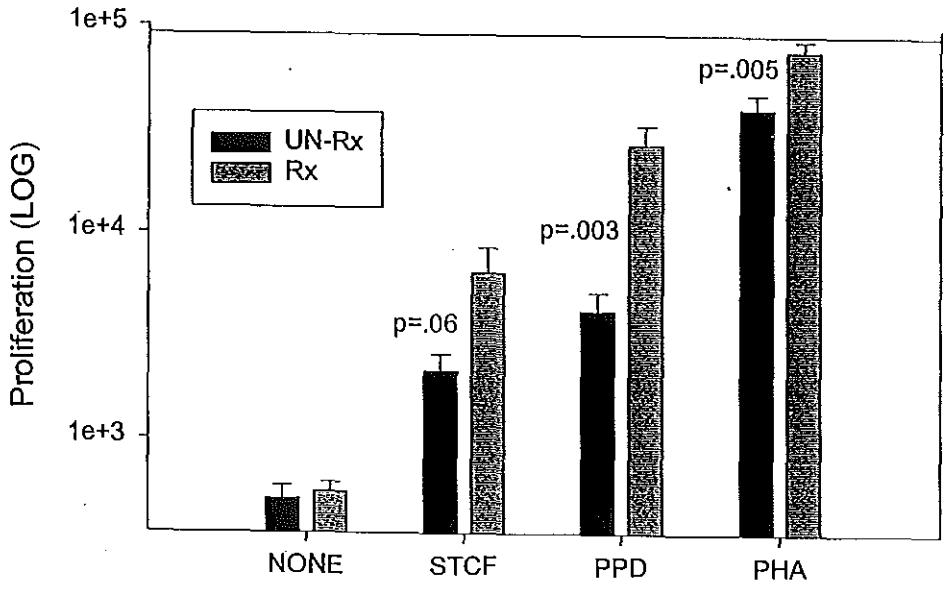

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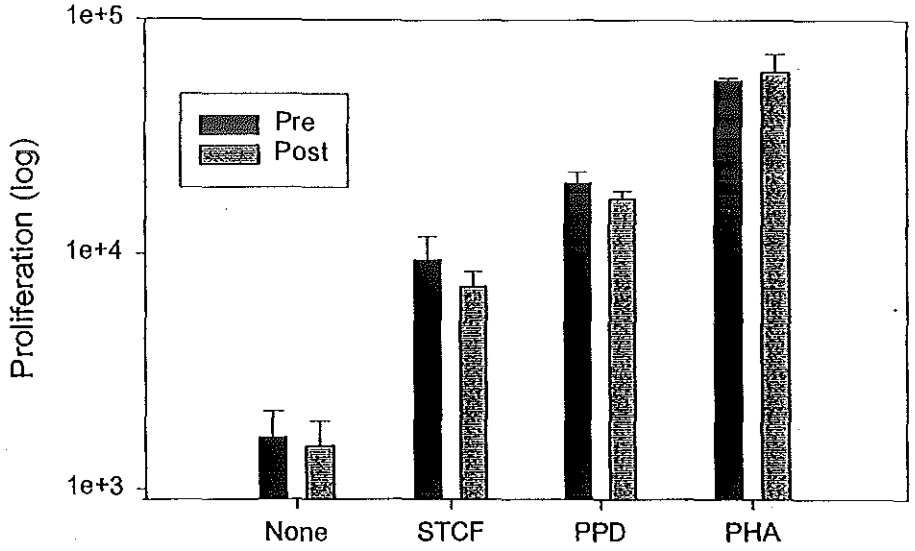

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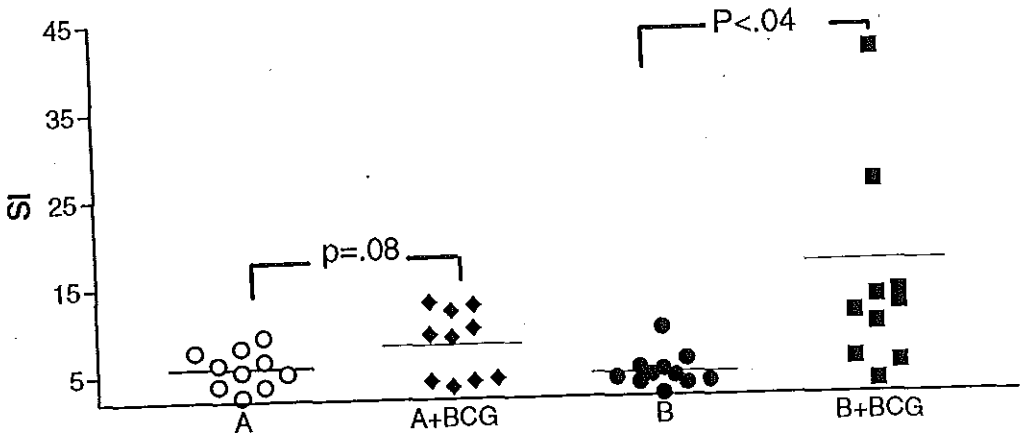

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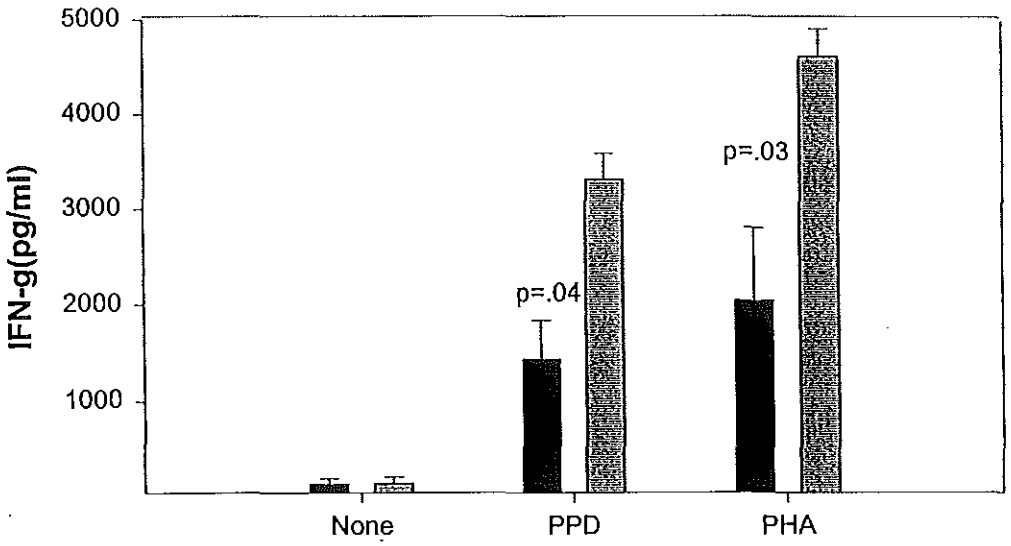

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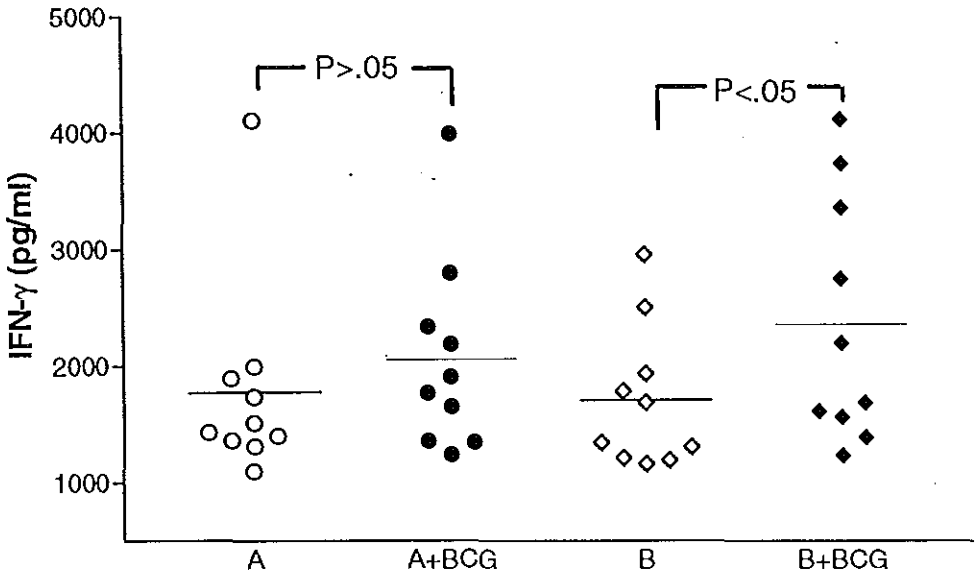

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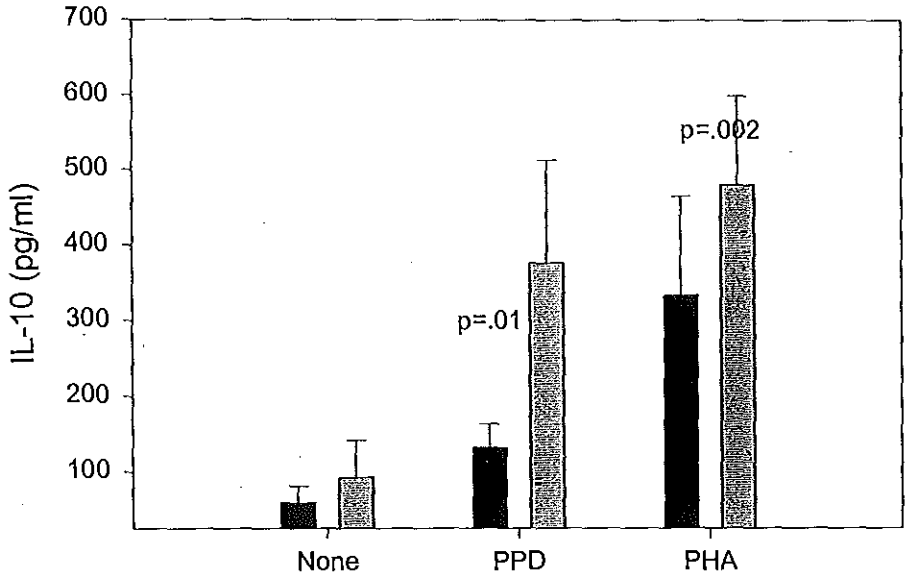

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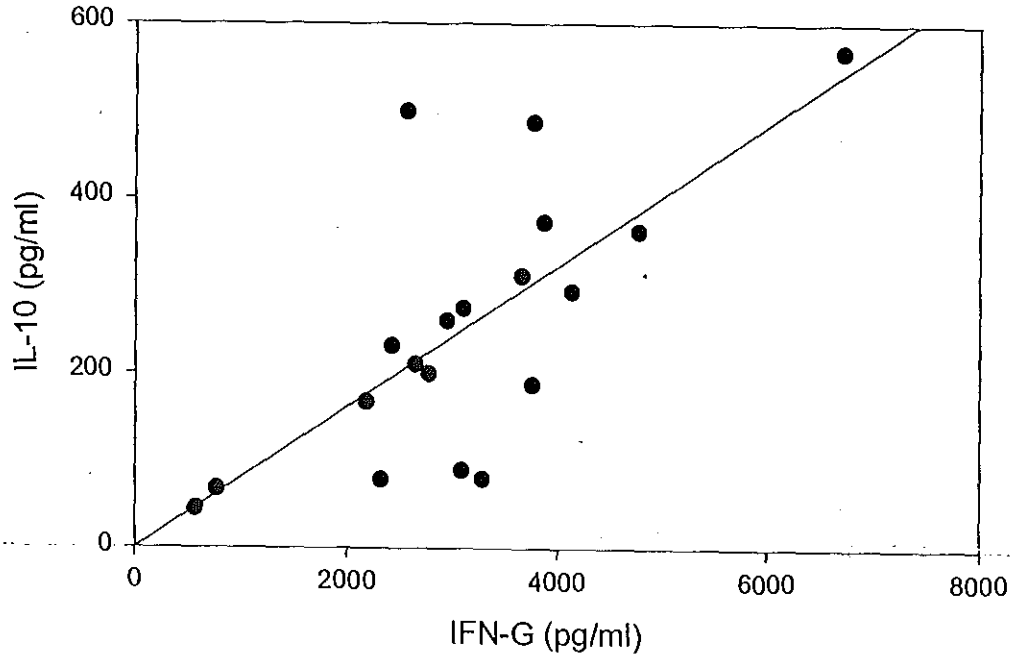

Fig-10 Relationship between IFN-g and IL10. There is a good positive correlation between the two cytokines (r=0.607) N=19.

4. Discussion

An established immune response profile to a given infection may influence the immune responses mounted to an unrelated antigen or the outcome of a concurrent heterologous infection. This was demonstrated by Curry and colleagues (1995) where the cytokine and antibody production accompanying immune response to *Schistosoma mansoni* egg antigens *in vivo* were found to affect the outcome of an infection with *Trichuris muris*. When mice susceptible to infection with *T. muris* are co-infected with *S. mansoni*, they acquire the capacity to resolve *T. muris* infection. The situation may be similar in humans. Although the number of studies addressing this problem are small, analysis of data coming from some laboratories indicate that infectious diseases, especially helminth infections endemic in Africa and other developing countries, profoundly affect the host immune system. Such an altered immune background makes the host more susceptible to for example, HIV infection and less capable of controlling that infection once it is acquired (Bentwich *et al.*,1995; Bentwich *et al.*,1996; Anzala *et al.*,1995).

There are two modes of T cell activation, depending on the pattern of stimulation to which particular cells are exposed overtime:

- full activation leading to proliferation and often also to activation induced cell death (apoptosis)
- 'partial' activation resulting in selective expression of some cellular functions and also transient resistance to full activation (anergy) (Grossman and Paul, 1992; Goodnow,1996).

In HIV infection both forms of activation are induced (Bentwich *et al.*, 1998). Grossman (1993) and others postulated the concept of 'infectious' induction of anergy, in which cells responding to specific antigens in the presence of partially activated cells tend themselves to become partially rather than fully activated and thus are recruited into the anergic pool, so that the state of anergy is propagated by successive cycles of anergy induction and recruitment. In HIV infected individuals a large proportion of phenotypically activated cells from lymphoid tissues is resistant to full activation and will display a degree of anergy (Bentwich *et al.*,1998).

Resistance of $CD4^+$ T cells to full activation limits HIV replication, this is regarded as effective host response to chronic HIV infection and a general strategy against other chronic infections as well (Grossman *et al.,* 1993; Bentwich *et al.*, 1998). This is supported by the observations made in an Ethiopian immigrant population to Israel having a very high prevalence of several infectious diseases other than HIV (Nehmias *et al.,* 1993) who display significant markers of chronic immune activation, one of the causes of which is thought to be helminth infections (Bentwich *et al.*,1996). In this HIV^- population where there is a high infectious background, the activation was associated with remarkable changes in the distribution and phenotype of peripheral blood T cell populations similar to those described in HIV^+ patients (Mahalingham *et al.*,1993; Messele *et al.*,1999;Pollack *et al.*,1993).

In this study, we examined whether broad-spectrum antihelminthic treatment of helminth exposed individuals may affect cellular immune responses to mycobacterial antigens. A

reduction or elimination of intestinal worms resulted in a statistically significant improvement in T cell proliferation, IFN-γ and IL-10 production by PBMC stimulated with Purified protein derivative of *M. tuberculosis* (PPD), ST-CF and a mitogen, PHA (Fig. 4, 6 &7). A similar improvement in T cell responses to PPD was observed in filaria worm infected subjects after treatment with diethylcarbamazine (DEC), as reported by Sartono and colleagues (1995). Moreover, Soboslay *et al* (1994) has noted that ivermectin treated onchocerciasis patients showed an increased PHA induced T cell proliferative response compared to pretreatment levels.

The observation of non-specific immunosuppression in intestinal helmenthiasis was anticipated and it is in accordance with the concept of bystander suppression, gut stimulated cells release inhibitory cytokines in the circulation. These cytokines nonspecifically downregulate cells that recognize other antigens (Elson and Zivny, 1996). Similar results were obtained by Barsoum *et al* (1982) in human schistosomiasis infection where an enhanced response in lymphocyte proliferation was observed after specific therapy against schistosomiasis. Greene *et al* (1985) showed that onchocerciasis patients have a reduced *in vitro* response to PPD.

The functional impairment of T cells (reduced proliferation and IFN-γ production) in helminth exposed Ethiopians is in favor of the notion that chronic infections would result

in functional defects in T cells similar to, but to a lesser extent than, that occur in peripheral T cells from HIV infected patients (Vingerhoets *et al.*,1998).

In the absence of HIV infection or other cause of immunosuppression only about 10% of people infected with *M. tuberculosis* develop overt tuberculosis, while the remaining 90% mount effective immune response and as a result either clear the infection, or keep it at bay (Zumla and Grange, 1998). An effective immune response against mycobacterial infections is thought to be mediated by cooperative interaction between T lymphocytes and mononuclear phagocytes with key roles played by CD4 lymphocytes (Ellner, 1997). This interaction is dependent upon the interplay of cytokines produced mainly by $CD4^+$ T cells (Barnes *et al.*,1993).Thus, T cells $CD4^+$ T cells reactive to mycobacterial antigens that produce cytokines chiefly of the Th1 pattern and also are believed to be engaged in the killing of cells infected with *M. tuberculosis* (Orme *et al.*,1993).

Our findings suggest that antigen specific T cell responses to *M. tuberculosis* particularly the Th1 responses (proliferation and IFN-γ production) could be down-regulated in persons with concurrent helminth infection. The implication of this could be that individuals with chronic helminth infection might have reduced ability to defend themselves against mycobacterial infections. Indeed, epidemiological studies show that the prevalence of tuberculosis is the highest in the part of the world where helminthes are also highly prevalent (Raviglione *et al.*,1995).

This finding raises several issues relevant to vaccine strategies in which induction of Th1 responses and IFN-γ production are believed to be important in the expression of

protective immunity. Helminth and mycobacterial infections co-exist in many less developed countries of the world.

In addition, the initially PPD negative subjects were vaccinated with *M. Bovis* BCG and later tested for mycobacteria specific immune responses in *vivo* and *in vitro*. The results were that PPD specific proliferation of T cells and the production of IFN-γ was significantly improved in the dewormed group compared to pre-vaccination levels, whereas in the placebo group, the difference was not statistically significant. Although the number of vaccinees followed up to the completion of the study was limited, the findings may indicate that immunization with BCG sensitizes individuals without worms better than it does in those with chronic worm infection. Indeed, studies conducted to determine the protective efficacy of BCG vaccination show that the vaccine provides the least protection in developing countries such as Malawi and India (Ponnighaus *et al.*, 1992; Tuberculosis prevention trial, Madras,1980) where helminthes are also endemic. Contrary to this, the MRC trial in UK showed up to 80% protection against pulmonary tuberculosis. Most authors argue that this variability could be due to difference in environmental mycobacterial exposure. Indeed, Palmer and Long in the experiments done on guinea pigs have demonstrated that the protection imparted by BCG was dependent on prior exposure atypical mycobacteria (Palmer and Long,1966). Brown and colleagues (1985) showed that exposure of mice to *M. vaccae* in drinking water before BCG vaccination influenced the ultimate responsiveness of spleen cells to mycobacterial antigens. Cheng and colleagues (1993) found that although peripheral blood monocytes from unvaccinated teenagers in the Chingleput trial area in south India (area where BCG

confers no protection against tuberculosis) were more efficient at inhibiting *M. microti in vitro* than were monocytes from their unvaccinated counterparts in England; and BCG vaccination enhanced this capacity to a greater extent in the English vaccinees. This was interpreted as evidence that the south Indian vaccinees had greater natural immunity against mycobacteria, attributable to their greater exposure to environmental mycobacteria, and that BCG does not add much because of this higher baseline due to prior heterologous immunity, genetic, socioeconomic, and other differences not withstanding.

The evidence for the involvement of environmental mycobacteria in naturally derived immunity to tuberculosis particularly in tropical countries contrasts with the fact that tuberculosis is most prevalent in those same areas where natural immunity should be most wide spread (Fine,1994). However, environmental mycobacteria are by no means the only influence on tuberculosis epidemiology. In fact an overview of published literatures by Colditz *et al* (1994) concluded that environmental mycobacterial exposure could explain only 41% of the observed variation between vaccine efficacy trials.

An alternative explanation which may account for part of the variation and for which we have some data is that the areas where the vaccine conferred the least protection (Tropical countries) are characterized by a high endemic prevalence of chronic infectious diseases (Hori *et al.*,1993). The host confronted with the infectious burden would be expected to mount a prolonged immune response to this challenge which would alter the normal immune balance (Bentwich *et al.*,1996). Bentwich and colleagues (1995) suggest that

changes in the host immune response may be caused by endemic infections, particularly helminthes. The fact that Albendazole treatment enhanced mycobacteria antigen specific T cell proliferation and IFN-γ production lays a ground for these assertions. Thus, the fact that BCG confers the least protection in areas where there is a high prevalence of helminthes may be because the baseline immunity in such areas is perturbed and such effects may have a bearing on the immune response to subsequent mycobacterial exposure.

Another interesting observation in this study was that BCG vaccination in our study population caused skin test conversion in 95% of the vaccinees 6 weeks after vaccination. However, only 25% retained skin test reactivity 4.5 months later. This may indicate that BCG vaccination does cause skin test conversion but vaccine induced tuberculin skin reactivity rapidly wanes with time. The result, if confirmed by a larger study would have an important implication because the Mantoux test is used in many countries as a diagnostic tool for exposure to *M. tuberculosis* but its use is limited in BCG vaccinated population. However, since vaccine induced tuberculin reactivity seems to be short lived (Fig.3), the use of the test can be extended to BCG vaccinated population. But one has to first know the approximate duration at which BCG induced tuberculin skin test reaction disappears. Our finding indicate that 6 weeks after vaccination almost all individuals (95%), initially PPD negative turned positive but 4.5 months later only 25% of the vaccinees retained it.

An additional observation in this study was that the group that produced higher level of IFN-γ also produced higher amount of IL-10, in agreement with previous report by Sartono and colleagues (1996) that T cells when cultured with PPD secreted high amount of IFN-γ and IL-10 at the same time. Moreover, it was shown that substantial amount of IL-10 can be produced by either subsets of $CD4^+$ T cells (Th1 or Th2) in humans (Del Prete *et al.*, 1993). Mosmann and Sad (1996) also indicated that the same cell that produces IFN-γ in humans could produce IL-10. Monocytes are a rich source of IL-10 and might contribute to the IL-10 release as a result of activation by IFN-γ produced by T cells. Since the human immune response to infectious agent is often a double edged sword that contributes to elimination of pathogens at the same time may also be harmful to host cells, the raised IFN-γ (pro-inflammatory cytokine) must somehow be controlled to minimize the inflammatory response and subsequent tissue damage; this may be why there was a corresponding rise in IL-10. This notion is also supported by the observations of Barnes *et al* (1993) who demonstrated that both IFN-γ and IL-10 were found to be raised in tuberculous pleural fluid, a mycobacterial infection representing a self limiting spectrum.

In summary, the alterations in immune responses to a non-parasite antigen that was observed in a murine helminth model have also been found to occur in humans. Humans infected with the helminth *S. mansoni* have been shown to have impaired *in vitro* production of IL-2 and IFN-γ in response to a mitogen (Zwingenberger *et al.*,1989; Zwingenberger *et al.*,1991). The present study shows that dewoming of a helminth exposed population enhances T cell proliferation and IFN-γ production in response to

mycobacterial antigens and a mitogen. Thus, although the problem calls for further study, the results presented here underline the importance of intercurrent infections and immunizations during human worm infections. Chronic helminth infected individuals may have an increased susceptibility to infections normally cleared by Th1 dependent immunity as well as altered immune responses to vaccines directed against intracellular infections.

The improved T cell response to mycobacterial antigens after deworming in BCG vaccinated individuals noted in this study, agrees with altered antibody responses to hepatitis B vaccine in schistosome infected humans noted by Ghaffar and colleagues (1990). The compromised response to hepatitis B vaccine in babies born to mothers with intestinal schistosomiasis reported by Bassily *et al* (1987) further supports this contention.

In conclusion, our data indicate that there is a functional change in peripheral T cells of helminth exposed population and that such changes can be reversed in a relatively short time following chemotherapy directed against helminths. Moreover, the improved mycobacterial antigen specific cellular response in the BCG vaccinated persons after deworming may indicate the importance of such infections in the efficacy of the vaccine in worm endemic parts of the world. Control of helminth infections in developing countries may deserve attention as such approaches may modulate the base-line immune response and lead to enhanced ability to cope with subsequent infections. Extensive investigation of the factors responsible for the compromised efficacy of BCG vaccine is

recommended as we are at present very far away from the realization of a better and new vaccine against tuberculosis, the world's major killing disease.

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