FIRST DR. C.M. SINGH ORATION LECTURE

on

RECENT ADVANCES ON IMMUNOLOGY AND IMMUNOPATHOLOGY OF MYCOBACTERIAL DISEASES

Delivered at
Indian Veterinary Research Institute, Izatnagar (UP)

on
10th January, 2005

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INTRODUCTION

Out of more than 50 known mycobacterial species which comprise the genus Mycobacterium, the majority are nonpathogenic environmental bacteria remain in soil and water. A few species which very successfully invade the host and cause pathogenicity are *Mycobacterium tuberculosis, M. bovis, M. leprae, M. ulcerans, M. avium* complex including *M. avium* subspecies *paratuberculosis.* As these organisms are very efficient in dampening the defense machinary resident macrophages and dendritic cells of the host by neutralising the antimicrobial properties of cell along with downregulation and upregulation of some of their genes, therefore, these are very efficient in growing inside macrophages. Inspite of this host defense builds up its complex machinary of innate and adaptive immunity and makes an effort to compartmentalise the growth of the organisms in immune structures called granulomas. As a result of this mycobacteria generally shuts off its genes to a dormant state which leads to a chronicity of infection. However, in such a situation, most of the time the host may remain asymptomatic with the potential to reappear later in an immunocompromised situation of the host due to other concomittent infection like AIDS. The present review will deal with only the aspects of immunology and immunopathology of mycobacterial infections.

Mycobacterial diseases, chronic granulomatous infections of humans and animals are responsible for worldwide mortality and morbidity of the host. While human tuberculosis is a major health problem causing about 3 million deaths anually in the whole world1, bovine tuberculosis, is a major cause of economic loss in countries including India where it is endemic and is probably responsible for zoonotic disease problem. *M. tuberculosis* complex consists of *M. tuberculosis, M. africanum, M. canettii, M. bovis and M. microti*. While *M. tuberculosis* is a major cause of tuberculosis in humans , in several parts of Africa *M. africanum* is the primary cause of infection2.The pathology and course manifested by *M. tuberculosis, M. bovis and M. africanum* in humans are similar. *M. microti* is a pathogen of voles but avirulent in humans.

M. marinum one of the closest to *M. tuberculosis* complex organism3 is now being used extensively as a model for *M. tuberculosis* pathogenesis[4-7].

M. ulcerans, the causative agent of Buruli ulcers, commonly noted in certain areas of the tropics, mostly in West Africa. This organism unlike other mycobacterial species secrete a cytotoxic polyketide toxin which causes extensive, painless, necrotic, noninflammatory ulcers in which bacteria grow extracellularly[8].

M. leprae, causative agent of leprosy of humans, manifests a wide clinical spectrum between two polar forms, tuberculoid and lepromatous leprosy[9].

The *M. avium* complex (MAC) is a group related to enviromental mycobacteria including *M. avium* subpspecies *avium*, *paratuberculosis* and *silvaticum* and *M. intracellulare*[10,11].

TUBERCULOSIS

Pathological Manifestations

The epidemiological study and natural history of the disease reveal that most of the hosts are resistant to the infection and can mount a strong immune response to *M. tuberculosis* infection. It is only 10 to 30% of individuals get infected. Of those who get infected 90% or more do not develop disease. In individuals where disease is manifested is mainly due to reactivation of latent infection.

M. tuberculosis is capable of evading innate and adaptive immunity in immunocompetent hosts and evoke sufficient lung pathology for transmission of infection by aerosol for a long period of time. It initially begins with accumulation of macrophages and neutrophils at the site of bacterial lodgment leading to a granuloma formation with epithelioid cells and giant cells around macrophage -laden bacterial growth[12]. The granuloma can later undergo caseous necrosis resulting in cavity formation. The host immune defense has a tendency to restrict the growth by laying down a fibrotic layer surrounding the granuloma, which produces microscopic nodules called 'tubercle'. If the host immunity is uphold then this tubercle gets fibrosed and calcified[12-14].

Interaction between Macrophage and Mycobacteria

Cell mediated immunity (CMI) is primarily expresed by initail macrophage mediated phagocytosis. Although the precise mechanism by which macrophages kill mycobacteria are not clearly understood but several studies have indicated that these cells get activated and gear up their antimicrobial function very efficiently. ***Phagosome - lysosome fusion:*** The lysosomal vacuoles are loaded with active hydrolytic enzymes capable of degrading a wide range of large molecules and microbes. These enzymes function at an optimal acidic pH (4.5-5.0)[15] in the intralysosomal environment. Phagosome containing bacteria after maturation fuses with lysosome forming phagolysosome[16-17]. Usually, organisms after an interaction with lysosomal hydrolases undergo extensive degradation within 2 hours of phagocytosis[17]. It was shown later that *M. tuberculosis* can prevent phagosome-lysosome fusion resulting in its survival and growth inside macrophages[18-19]. It was further noted that viable *M. tuberculosis* containing phagosomes do not fuse and consequently viable mycobacteria are able to grow inside macrophages. Later it was revealed that mycobacterial sulfatides[20], derivatives of multiacylated trehalose 2-sulphate, alysosomotropic polyanionic glycolipid [21-22] are responsible for phagosome-lysosome fusion. Later, in vitro studies showed that *M. tuberculosis* are capable of generation of copious amounts of amonia in cultures (up to~20mμ) which has been thought to be inhibitory for phagosome-lysosome fusion[23]. Supporting evidence for this also showed that amonium chloride has inhibitory effect on saltatory movement of lysosomes and has ability to alkalanize intracellular vacuoles[23-24]. However, finding of the ability of other bases to enhance phagosome-lysosome fusion[23].Contradicted the above finding and therfore the exact mechanism of phagosome-lysosome fusion prevention by amonia remains to be confirmed again. Mycobacterial enzymes, urease which catalyzes urea to amonia and carbon dioxide and glutamine synthetase which has a role in alteration of amonia level through nitrogen metabolism have been under investigation for intracellular survival of *M. tuberculosis*[25-27].

The RabGTPases are large family of proteins with a variety of regulatory functions in membrane traffic of various endocytic compartments. Mycobacterial phagosomes retain Rab5, which plays a role in interaction between early endocytic compartments and phagosomes and does not allow the phagosome to mature[15-16]. It was further noted that *M. tuberculosis and M. avium* containing phagosomes can block the vacular ATPase proton pumps and result into reduction in acidification[28-30]. It was later found out that two host proteins, one associated with mycobacterial phagosome; coronin 1/TACO (tryptophan-aspartate-containing coat), an actin binding protein-a component of host phagosome required for phagocytosis and Gal3/Mac2, a protein expressed on the macrophage cell surface and associated with the Golgi and nucleus[33]. These proteins are involved in regulating trafficing of molecules in phagosomes. After phagocytosis of mycobacteria these proteins are retained in the phagosome and prevent their maturation and phagosome -lysosome fusion.

Role of Reactive Oxygen Intermediates (ROI) and Reactive Nitrogen Intermediates (RNI): ROI has been shown to be released by activated macrophages induced by cytokines like IFN-?, TNF-a and these molecules are capable of killing microbes in these macrophages. However, mycobacteria are capable of evading the toxic effect of ROI by several means. Lipoarabinomannan (LAM) and phenolic glycolipid-I (PGL-I), components of M. tuberculosis and M. leprae are potent scavangers of oxygen radicals[34-35]. Further sulfatides have been shown to interfere with the oxygen mediated antimycobacterial killing by macrphages[14]. On the other hand, toxic RNI liberated by activated macrophages have been shown to inhibit the growth of M. tuberculosis both in vivo and in vitro specially in mice[36-40].

Role of Toll-like Receptors (TLR): The human TLR is a transmembrane protein with a leucine-rich repeat in extracellular domain of cell. Its cytoplasmic domain is homologous to that of IL-1 receptor. Constitutively active human TLR when transfacted in a cell it induces activation of NF-κB pathway leading to cytokine liberation and co-stimulatory molecule expression[41]. The TLR-dependent

activation of the NF-?B pathway is mediated by an adapter protein, MyD88[42]. For an effective activation and liberation of IFN-r macrophages require MyD88 adapter protein. MyD88-/- macrophages are five 5 to 100 fold less effective in activating many genes like, nitric oxide synthase, IRG-1, IP10,MIG, RANTES. In vitro studies using M. tuberculosis have shown strong evidence that it can signal through TLR2 and TLR4. It has been shown while a soluble heat- stable protease resistant component of M. tuberculosis can activate through TLR2, a heat-sensitive cell associated mycobacterial component activate through TLR4[43-44]. Recently, it has been noted that prolonged exposure of M. tuberculosis LprG (Rv1411c), a 24kDa lipoprotein, induces marked inhibition of MHCII antigen processing However, LprG was also found to stimulate TLR2 dependent TNF-a production[45].

Mycobacterial Entry Into Macrophages

In vitro assays using cultured macrophages have shown that the pathogenic mycobacteria can use a variety of receptors to gain entry for their survival and safety inside a cell[46]. It was recently shown that requirement of cholesterol appeared specific for mycobacterial entry[48], however, whether cholesterol is essential for direct binding with mycobacteria or allowing an appropriate receptor interactions have yet to be determined[49-50].

A pivotal role played by macrophage has long been understood in tuberculosis from histopathological and experimental studies[51]. In rabbit and mouse models of pulmonary tuberculosis it has been clearly shown that macrophages are responsible for phagocytosis and dissemination of *M. tuberculosis* to the deeper tissues[51-53]. Recently, infected macrophages with *M. marinum* have been found to migrate from blood stream or hind brain ventricle to deeper tisues in zebrafish. It was also observed that uninfected macrophages from distant areas can engulf dead infected macrophages[54]. Recent comparative gene expression studies using microarray between *M. tuberculosis* log phase culturees and *M. tuberculosis* grown in vitro inside macrophages revealed differential expression of a total of 601 *M. tuberculosis* genes within macrophages relative to to that of

axenic cultures. This study further showed that stimulation of infected macrophages with IFN-γ upregulates an additional [68] activation specific genes in response to generation of NO by macrophages[55].

Granuloma Formation

After the entry of mycobacterium it is engulfed by macrophages or dendritic cells and are transported to deeper tissues by macrophages and phagocytic cells and additional macrophages start gathering around. Tuberculous granulomas in humans, animals and mice have large component of T lymphocytes, some B lymphocytes, dendritic cells, neutrophils, fibroblasts and extracellular matrix components[14-56]. CD4+ and CD8+ T lymphocyte subsets play an important role in maintenance of granuloma. The macrophages differentiate into epithelioid cells and in several situations macrphages fuse to become giant cells. A common feature of granuloma is the occurance of caseation at the centre of granuloma[55] surrounded by epithelioid cells with a peripheral cuff of lymphocytes (CD4+ and CD8+ Tcells)56,57. Mycobacteria mainly reside in the macrophages and in large number in the central caseous zone. In humans and animals the caseous area may become fibrotic and calcified. In a given host at a given time point granulomas can be at different stages of evolution[58]. However, the actual mechanism of caseation still remains unclear. Although, mice infected with *M. tuberculosis* form granulomas without caseation but in rabbits and guineapigs caseations do occur[59-61].

Immunoregulatory Molecules

Cytokines are resposible for immune functions by the host against the pathogen. *M. tuberculosis* also induces cytokine mediated response for inducing immunity against the infection by the host. The role of the cytokines and their involvement in host response are given as below:

Interleukin-12 (IL-2): M. tuberculosis infection induces the host to gear its T helper -1 (Th1) type of immune response. Macrophages and dendritic cells after engulfing *M. tuberculosis* liberates IL-12[59,61,62] which ultimately leads to IFN-γ liberation and development of Th1 immunity[63]. It has also been shown that early administration of IL-12 in infected

BALB/c mice significantly lowered bacterial growth[64]. Further, it was shown that IL-12p40 gene deficient mice were susceptible to M. tuberculosis infection[65]. A recent study indicated that administration of IL-12 DNA could substantially reduce bacterial burden in mice with chronic *M. tuberculosis* infection[66].

Interferon-gamma (IFN-γ): IFN-γ is the key cytokine in the Th1 immune response and is essential for the control of *M. tuberculosis* infection in lung[14,67]. In tuberculosis IFN-γ is produced by both CD4+ and CD8+ Tcells[68-70] and by NK cells [14]. In the murine system, IFN-γ strongly induces iNOS gene transcript mRNA expression resulting into enzyme activity[71-72]. It has been further noted that IFN-γ knockout mice are most suceptible to virulent *M. tuberculosis* infection[73-74]. In human situation where individuals were noted to have defective IFN-γ gene or its receptor were found to be prone serious *M. tuberculosis* infection and other mycobacteria[75]. However, IFN-γ response has been noted in both PPD+ subjects and in active cases of tuberculosis. It has been further noted that the levels of this cytokine is lowered in patients patients of active tuberculosis[76-77]. In addition it was shown that IFN-γ ultimately is also able to prevent macrophages for production adequate quantum of IFN-γ indicating the limitation of this cytokine in containing *M. tuberculosis* growth in lungs[78]. Recently, it has been shown that aerosol challenge by IFN-γ upregulated only 15 genes of the bronchoalveolar macrophages of tuberculosis patients including IP10 and MCP1[79]. It has already been established that *M. tuberculosis* infection induces IRF-1 (IFN regulating factor1)[80-81] gene which has pleiotropic function on the immune system[82] however, the nature of this regulation is still not known. A recent study demonstrated that *M. tuberculosis* inhibits RNA export from the nucleus and affect IRF-1 expression leading to pathogenicity. On the other hand, IFN-γ synergistically stimulates the infected THP-1 cells and upregulates the function of IRF-1[83].

Interferon-alpha (IFN- α): It has been reported earlier that IFN-α /ß system helps host defense against *M. tuberculosis* infection in mice[84] and the infection induces human

macrophages and dendritic cells for the production of IFN-α/ß [85-86]. Futher aerosolized IFN-α in combination with chemotherapy has been shown to improve the clinical condition of tuberculosis[87]. Later it was noted in vitro culture system that IFN-α/ß released by *M. tuberculosis* infected macrophages inhibit the tyrosine phosphorylation of signal transducer, STAT-1 indicating that it is cellular signal transduction and associated with pathogenicity[88]. Therefore, further research is needed to find out the exact role of IFN-α in immunomodulation of tuberculosis.

Interleukin-4 (IL-4): IL-4, inducer of Th2 response, has not been found at a very high level in tuberculosis patients by most of the authors[76-77] however, it has been found to induce apoptosis of *M. tuberculosis* reactive T cells in presence of TNF-α[89]. *In situ* demonstration of elevated levels of IFN-α expression in granuloma of lymph nodes of patients with pulmonary tuberculosis and tubercular lymphadinitis have been noted with low levels of IL-4 mRNA expression[76]. In experimental situation in gene knockout or ILp40-/- mice no shift to Th2 was observed[74,65]. Even when granulomas were investigated for IL-4 expression by *in situ* hybridization in advance tuberculosis 3 out of 5 patients showed presence of IL-4 but never in absence of IFN-γ expression[90]. From the above it is clear that IL-4 levels do not correlate with clinical status of tuberculosis.

Tumour Necrosis Factor-alpha (TNF-α): The cytokine, TNF-α plays important roles in both immunity and pathogenesis of tuberculosis. *M. tuberculosis* is known to induce macrophages, dendritic cells and T cells for secretion of TNF-α[91-94] which control *M. tuberculosis* infection. Mice deficent of TNF-α or TNF-α receptor are more susceptible to M. tuberculosis infection than normal mice[95-96]. TNF-α along with IFN-γ is known to induce expression of NOS2[72,90,97]. Further sufficient data have been accumulated to establish the importance of this cytokine in granuloma formation in tuberculosis and other mycobacterial diseases[96-100]. In murine model deficiency of TNF-α leads to a reduction in granuloma formation with fewer epitheliod cells[95] and impaired

colocalization between lymphocytes and macrophages[96]. However, several mechanisms of TNF-α which promotes granuloma formation are yet to be determined.

The other major function of TNF-α that it destroys the host tissue and leads to pathological progression of the disease in the lung[101-103]. Although TNF-α is responsible for tissue damage, it was noted that thalidomide treatment downregulated the cytokines TNF-α, IL-6, IL-10 resulting in a reduction in the size of the granuloma but did not bring about any change in the bacterial number[103]. However, it was later shown in mouse model that netralization of TNF-α resulted in 100% mortality and the lungs of the mice showed extreme involvement with disorganized granulomas, diffuse cellular infiltration and pathologic manifestations of chronic inflammation[14]. These observations rather suggested that TNF-α might play an important role in limiting the pathologic response in tuberculosis. Considering the above it is clear that further study is required for understanding the role of TNF-α in tuberculosis.

Interleukin-10 (IL-10): IL-10 is known as an antiinflammatory molecule which is known to neutrilize the function of proinflammatory cytokines. This cytokine during *M. tuberculosis* infection is produced by the macrophages and T cells of the host and downregulates IL-12 production resulting in decrease in IFN-γ level. IL-10 has been found to directly inhibit the CD4+T cell responses, as well as APC function of cells infected with mycobacteria[104]. Transgenic mice, constitutively expressing IL-10 were unable to clear BCG infection inspite of normal T cell responses with IFN-γ production[105]. Lastly, IL-10-/- mice were not found to be more resistant to acute *M. tuberculosis* infection as compared to wild type mice[106]. Further, the role of IL-10 involvement in pathology and protection are being worked out.

Interleukin-6 (IL-6): IL-6 is known to modulate immune response. It has been shown to suppress T cell responses to antigens in BCG infected macrophages[107]. In IL-6-/- mice early increases with decresed IFN-γ level compared to control mice following a low dose of aerosol infection has been noted[108]. Although, these mice with low dose of infection survived but

with high dose of M. tuberculosis IL-6-/- mice were susceptible to infection.

Transforming Growth Factor-ß (TGF-ß): TGF-ß is an antiinflammatory cytokine and has been responsible for suppression of T cell responses in tuberculosis patients[109]. *M. tuberculosis*[110] and LAM[111] induce human monocyte to produce TGF-ß which has been found in granulomatous lesions. This interleukin may also inhibit macrophage in producing NSO2 by IFN-γ[111]. Increased level of TGF-ß1 have been observed in the pleural fluid and plasma of tuberculous pleurisy patients[112-113]. TGF-ß has been used to induce pleurodesis in animal models and appeared to be a good candidate for use in humans[114]. However, an increased level of TGF-ß have been associated with increase severity of tuberculosis. PBMC from patients with advanced pulmonary tuberculosis produced significantly higher levels of TGF-ß in th absensce or presence of whole sonicated antigen of *M. tuberculosis* than PBMC from patients with mild to moderate disease and PBMC from healthy control patients[115].

T Cell functions during *M. tuberculosis* infection

Status of CD4+ T cells: Experimental models in mice established that CD4+ T cells are primarily responsible for protection against *M. tuberculosis* infection[116]. Anti-CD4+ antibody depletion of CD4+ T cells[117], adoptive transfer [116,118] or the use of CD4 gene deleted mice[119-120] strongly established that CD4+T cells are essential for controling infection. In humans important role of CD4+ T cells established from the cases of reactivation of tuberculosis in HIV infected individuals in which CD4+ cells unndergone reduction[121]. Further, in HIV-infected patients with tuberculosis, mycobacterial load increases as CD4 depletion becomes more prominent[122]. In addition, a paucibacillary *M. tuberculosis* infection, tuberculous pleuritis, a self-resolving disease, is associated with proliferation of CD4+ T cells with high levels of IFN-γ[123]. The mechanism by which CD4+ T cells contribute to protection against *M. tuberculosis* is due to induction of Th1 cytokines, IL-2 and IFN-γ[124] which are sufficient to activate macrophages for intracellular killing of the organism. In MHC II-/- or CD4-/- mice, levels of IFN-γ is severly lowered

during the early stage of infection. Further experiments in mouse model indicated that when with a low dose *M. tuberculosis* infection a chronic infection is generated, treatment with anti-CD4 antibody can evoke reactivation of the infection leading to a high bacterial burden in the lungs causing death of mice[125]. However, IFN-γ levels of CD4-depleted and control mice were same due to IFN-γ production by CD8+ T cells. Further, there was no change in the NSO2 production or activity in the CD4+ T cell depleted mice indicating that there are IFN-γ and NOS2 independent CD4+ T cell dependent mechanisms for control of infection.

Priming of CD4+ T cells, a major function of dendritic cells *in vivo*, is less likely to be affected by *M. tuberculosis* because it was noted that there was no reduction in the levels of MHCII on the cell surface after *M. tuberculosis* phagocytosis[92,96]. This is further supported by the fact that a strong CD4+ T cell mediated immune response could be generated during *M. tuberculosis* infection. However, a defective T cell stimulation may be contributed by by APCs due to production of cytokines like TGF-ß, IL-6 or IL-10 as mentioned earlier.It is very clear that *M. tuberculosis* is equipped with immune evasion stratigies by underexpression and overexpression of certain genes when it is engulfed by macrophages or dendritic cells[55].

Status of CD8+T cells: Experiments in mice definitely proved the protective role of CD8+ T cells[127]. MHC class I presentation of several cytoplasmic antigens is very efficient in tuberculosis. Mice genetically deficient in ß2 microglobulin or TAP in CD8+ T cells were quite susceptible to *M. tuberculosis* infection[127-129]. Recent studies in mice demonstrated migration of CD8+ T cells to the lungs like CD4+ T cells following *M. tuberculosis* infection[93,130]. These cells are able to produce IFN-? and lead to lysis of infected macrophages[93,127,130]. These interactions between CD8+ T cells and infected macrophages may be manifested in class I restricted or non class I restricted fashion.

Class I restricted CD8+ T cells: In humans and mice, several antigens, for example, 38kDa 131, 65kDa-heat shock protein 132 and 19kDa[133] have been shown to be recognized by CD8+

T cells in MHC class I restricted manner. These T cells have been further shown to secrete IFN-γ with lysis of infected macrophages[14]. Although the exact mechanism of gaining entry into the cytoplasm of APCs is not clearly understood, however after entry of mycobacteria in the macrophages, the bacilli have been noted outside the phagosome after 4 to 5 days of phagocytosis[134] and and the prsentation of the antigen by these infected macrophages can begin at 12 hr which can be later recognized by T cells[135]. It has been shown that live but not heat-killed *M. tuberculosis* infection of macrophages facilitated MHC class I presentation of soluble ovalbumin in a TAP-dependent manner indicating that ovalbumin taken up by phagosomes along with *M. tuberculosis* leaked into the cytoplasm[136]. Further, in BCG infected macrophages proteins up to 70kDa could be noted in the cytoplasm[137].

Nonclassically restricted CD8+ T cells: CD1 molecules are nonpolymorphic antigen presenting molecules which have been grouped as Group I (CD1a, b and c) and as Group II (CD1d), CD1d molecules like MHC1a molecules, present lipids/glycolipids to T cells[138]. CD1 restricted T cells may be either CD4-8-, CD8+ or CD4+ T cells[139]. Several mycobacterial antigens, such as, mycolic acid[140], LAM[141], phosphatidyl inositol manoside[142], glucose monomycolate[143] and isoprenoid glycolipids[144] have been shown to be presented to the T cells by CD1. Recent study shows that mycobacterial lipids within the phagosomes can be transported in and exported from the cell to endocytic vesicles and can be taken up by any bystander cells for presentation to T cells[145]. Although the exact role of CD1 restricted cells in innate and adaptive immunity is yet to be found out, initial studies indicate that CD1 restricted response could be generalized from PBMC of both PPD+ and PPD-subjects[140,146]. Another study using isoprenoid glycolipid as an antigen could note PBMC proliferation from PPD+ and not PPD-subjects[140]. This proliferation further could be blocked by anti-CD1c antibody [143]. CD1 molecules are found usually on dendritic cells with low level of expression on macrophages[147]. It is possible that dendritic cells which are in majority in lungs might be involved in stimulating T cells in CD1 restricted

manner in the granuloma. Dendritic cells can be infected with *M. tuberculosis* and it is possible that these cells are playing a pivotal role in protection against tuberculosis. Therefore, research using dendritic cells are needed to unravell various mechanisms which are involved in protection against tuberculosis.

Effector function of CD8+ T cells and protective immunity: The effector function of CD8+ T cells are primarily expressed by production of IFN-γand cytotoxicity of the infected cells. Whether both of these functions are conferring protection in tuberculosis is largely unknown. However, it is known that specifically sensitized CD8+ T cells liberate IFN-γ upon TCR engagement or by interaction with *M. tuberculosis* infected dendritic cells in mice[93]. However, the quantum of IFN-γ produced in such a situation was much lower than CD4+ T cells. Therefore, CD8+ T cell interaction with sensitized macrophages/dendritic cells needs further study. The function of CD8+ T cells in causing lysis of infected cells via perforin and granzymes or the Fas/FasL pathway. Reduction in intracellular mycobacterial number was noted by observing lysis of infected human dendritic cells and macrophages by CD4- MHC classI restricted CD8+T cells[148-149]. It was further noted that the perforin was responsible for killing of intracellular mycobacteria[148].In another study it was noted that perforin was responsible for forming pores and the cytotoxic molecule, granulysin was required for lysis of mycobacteria[149].

Status of γδ T cells: Increasing evidence is being accumulated regarding the role of γδT cells in immunity against tuberculosis[150]. These cells recognize a wide range of antigens including the unprocessed protein antigens[151]. Although studies in mice already indicated the important role of γδT cells in early protection[152-154] such observations in humans and animals have not yet been established. However, higher frequency of antigen-specific γδ T cells especially bearing Vγ9 Vδ 2 cells[155-156] having their ability to produce IFN-γ and
cytotoxicity have been recognized in protection against tuberculosis[157].

BOVINE TUBERCULOSIS

It is predominantly caused by *M. bovis*, is a major cause of economic loss in countries where it is endemic and in some countries it has a significant zoonotic problem[158].

The mechanism of pathogenesis and granuloma formation is similar to *M. tuberculosis* in man which has already been detailed above.

It was noted by several workers that IFN-γ response is the major interleukin reponse in infected cattle[159-162]. When both IFN-γ and IL-4 responses to the antigenic challenge (ESAT6) were monitored then it was noted that the IFN-γ level was at a high level all throughout the disese process. On the other hand IL-4 response was delayed and on the 8th week after challenge there was a peak response which declined later.However, both IFN-γ and IL-4 levels were able to discriminate between infected and noninfected cattles in the field in the devoloped countries[163]. Utilizing these interleukin responses no data are available from the underdeveloped and developing countries.

JOHNE'S DISEASE

Johne's disease, also called paratuberculosis, is a chronic granulomatous enteritis of ruminant animals caused by *M. avium* subsp.*paratuberculosis*. Although Johne's disease can cause death of the animal however, due to the chronicity of infection the morbidity of animal is much more and therefore the disease lay significant impact on the economy of the country[164-165].

Disease Progression and Immunopathology

The disease typically progresses through three distinct stages[166]. First and second stages are called subclinical stages where animals are generally asymptomatic. In the second stage also animals remain asymptomatic without developing clinical disase or might progress to clinical stage after 3 to 4 years of infection. In the first subclinical stage the animal is infected but shedding of bacilli in the feces cannot be detected. In the second subclinical stage, the intermittent excretory phase, the bacilli may be discharged in the feces intermittently and not continuously. The third stage is the clinical excretory phase which is characteized by persistent shedding of high numbers

of bacteria in the feces with severe diarrhoea associated with weight loss.

M. avium subsp.*paratuberculosis* enters tissue through M cells present in the distal ileum of calves and goats[167-168]. In the Peyer's patches in the intestinal tracts and the jejunum of ruminants adaptive immune response is initiated with T and B cell responses[169].

Fibronectin attachment proteins (FAPs) are family of fibronectin (FN)-binding proteins present in several species of mycobacteria[170-174]. Attachment and internalization of *M. bovis* BCG, *M. leprae* and *M. avium* subsp. *paratuberculosis* by epithelial cells *in vitro* has been shown to be dependent on the interaction between FAP and FN[172,175,176]. B1 integrins have been identified as the host cell receptor for FN-opsonized mycobacteria *in vitro*[174-175]. M cells are unique which express high density B1 integrins on the luminal surface[177] and FN bound by organisms simply explains the portal of entry through M cells. Recently, using gut loop assay technique in mice the specificity of M cell-entry through formation of FN-bridge formed between FAP-of *M. avium* subsp. *paratuberculosis* and inegrins of M cells have been demostrated[178].

Lesions associated with the disease is often diffuse and granulomatous and are typically restricted in the ileum and particularly to the ileocecal valve region of small intestine[179]. The pathology is mainly due to severe immue pathology induced by immune cells and chronic inflammation[180-181]. Draining lymph nodes near the lesion often exhibit hyperplasia with large numbers of T cells and macrophages[180,182] in infected ileal tissues and in associated lymph nodes.

After the initial exposure to the organism, a proper T cell response characterized by the release of proinflammatory cytokines like IFN-γ, IL-1a, IL-6 along with production of IL-2 is generated by the host[183-184]. However, in most of the cases the disease progresses and the Th1 immune response gets shifted to Th2 type of response with IgG1 type of antibody response against the organism[184-186]. A recent study using cytokine gene expression in PBMC and tissues of infected cattle indicated that only in subclinically infected cattle IL-10 gene expression was consistantly

enhanced in PBMC after stimulation with *M. avium* subsp.*paratuberculosis*. In ileal tissues of infected cattle, expression of genes encoding IFN-γ, TGF-ß, IL-5 and IL-8 was greater than the expression in comparable tissues from control uninfected cattle. However, expression of gene encoding IL-16 was lower in tissues of infected than in control tissues. The draining mesentric lymph node tissues from the infected site showed higher expression of TNF-α,IL-8, IL-2 and IL-10genes than similar tissues from control animal. In contrast, genes encoding TGF-ß and IL-16 were expressed at lower levels in lymph nodes from the infected cattle than the uninfected cattle[187]. The above study suggested that cells or other mechanisms capable of limiting proinflammatory responses to *M. avium* subsp. *paratuberculosis* develop in Johne's disease to control tissue damage and immune responses.

LEPROSY

Leprosy , caused by *M. leprae*, is a chronic infectious disease inflicting human beings since the ancient times. As the world by now has attained the targetted prevalence of less than 1 per 10,000 population, therefore, the disease is at present is considered as a disease of no consequence. In India, the prevalence is still less than 3 per 10,000 and it is expected that by another few years the prevalence will come down to less than 1 per 10,000. However, in India still in certain states like Bihar,Uttar Pradesh, Madhya Pradesh, Orissa and West Bengal the prevalence rate is very high and inspite of multidrug therapy the new case detection rate is not going down indicating the existance of man to man transmission of the disease.

The Disease

The disease is expressed with a variety of manifestations depending on the immunity of the host. It may involve nerves (mainly peripheral nerves), skin and mucous membrane. The disease sometimes remain restricted to nerve only and then it is called as pure neuritic leprosy. Considering all the parameter like clinical, bacteriological, histological and immunological the disease has been classified in a spectrum of five-group system with two polar ends. In one end of the pole is

tuberculoid (TT) type and in the other end of the pole is lepromatous type(LL) of leprosy. In between these poles there are borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL) forms9.

It has been noted that at the TT/BT end the level of CMI towards *M. leprae* remains highest and as the disease progresses downwards in the spectrum towards LL pole the CMI towards *M. leprae* goes down as determined by lepromin skin testing and *M. leprae* specific lymphocyte proliferation assay. On the other hand, the level of anti-*M. leprae* antibody and bacteriological load go higher and higher with the advancement of the disease. In this disease there exists a clear inverse correlationship between CMI and humoral immunity as well as bacteriological load[188].

Characteristics of T- Lymphocytes in the Granuloma

Extensive studies of T lymphocyte populations have been performed by researchers using monoclonal antibodies for immunophenotyping of cells in the lesions. It was noted that while proportion of CD4+/ CD8+ ratio was more in TT/BT lesions in BL/LL leprosy the ratio was reversed[189-191]. It has been recently observed that although the CD4+/CD8+ ratio of 2:1 in blood and lesion is not altered in TT leprosy, T-memory (CD4+CD29+) and T-naïve (CD4+CD45+) ratio is 1:1 in blood and 14:1 in lesions[192].

***In-situ* Environment in Granuloma**

The pattern of distribution and localization of various immune cells in the granuloma and epidermal layer of skin help in understanding of the immunological microenvironment. In tuberculoid leprosy the CD4+ T cells remain scattered inside the granuloma along with macrophages, while CD8+ T cells remain in the mantle of the granuloma in the form of a ring[190,193,194]. It has been established that the above pattern of cell arrangement is an indicator of an immune granuloma. In tuberculoid leprosy, the presence of CD4+ cells, memory T cells and macrophages point towards an active immune interaction with activation and maturation of immune cells, leading ultimately to destruction of *M.leprae*. On the contrary, finding of prdominantly CD8+ cells, macrophages and a few CD4+ cells

inside the granuloma indicate generation of a suppresssive function in lepromatous leprosy. In addition to the above, the finding of more numbers of CD1+ Langerhan's cells in epidermis and the priphery of tuberculoid granulomas than in lepromatous lesions suggests an environment of effective immunity in tuberculoid leprosy (reviewed in191).

Th1/Th2 functions of lesional cells: Spectral manifestations in human leprosy have provided the immunopathologists a great scope in investigating the cytokine secretion profile to unravel a variety of immunological responses to the intracellulare parasite, *M. leprae* It has been clearly demonstrated that the majority of *M. leprae* responsive T cells generated from PBMC or skin lesions of TT patients produce IFN-γ, IL-2 and TNF-α but little or no IL-4, IL-5 and IL-6. Further, using immunoperoxidase techniques IL-2, IFN-r and TNF-α positive cells have been shown to be present in higher number in tuberculoid leprosy compared to that of lepromatous leprosy. Using *in situ* hybridization and mRNA-based PCR further proof for the presence of IFN-α, IL-2, TNF-α, LT and GM-CSF- producing T cells in TT leprosy has been documented. Contrary to the above, these interleukins were found to be at lower levels in BL/LL lesions. However, higher levels of mRNA for IL-4, IL-5 and IL-10 were present in BL/LL than in TT/BT lesions (reviewed in 191). This differential compartmentalization between Th1 and Th2 are almost similar to the dichotomy of Th1 and Th2 which exists in strains of mice[195]. Further, functional analysis of T cell clones from lesions revealed that CD4+ clones from tuberculoid leprosy liberating mainly IFN-γ and CD8+ clones from lepromatous lesions produced IL-4[196]. It was subsequently noted that some CD8+ clones from LL produced high levels of IFN-γ in addition to IL-4. It was also observed that IL-4 and IFN-γ coproducing clones gradually shifted to Th2 type, whereas non-IL-4 producing clones secreted very high levels of IFN-γ on prolonged cultures[197]. In addition when lesions from BL patients were analyzed, it was noted that Th1 type of cells produced IFN-γ and TNF-α with minimal IL-6 production, whereas Th2 type of cells produced IL-4, IL-5, IL-13, IL-10 and Th0 cells produced both types of cytokines[198].

Simultaneous measurement of memory T cell 1 (MT1, CD45RA-, CD62L-, CD11abright, IFN-γ biased) and memory T cell 2 (MT2, CD45RA-, CD62L+, CD11adim; IL-4 biased) of peripheral blood of leprosy patients showed that the ratios of MT1/MT2 differed significantly between tuberculoid and lepromatous leprosy patients[199].

Status of γδ and αß T cells

It has been observed that mycobacterial exposure leads to expansion of γδ T cells and γδ T cells have been found in the skin lesions of leprosy[200]. It was also noted that 25 to 30% of CD3+ T cells of both lepromin skin reaction and revarsal reaction are γδ T cells. The finding of proliferation of γδ T cell lines in expanded culture generated from skin lesions and peripheral blood in response to *M. leprae* sugests their role along with cytokines in granuloma formation[200].

CONCLUSION

Mycobacterial diseases are chronic diseases and mostly remain under control in a host expressing adequate immunity. It is only when there is lowering in the level of CMI (TH 1 type), the focus of containment of infection gets reactivated and the disease is clinically manifested. As the disease progresses there is a shift of Th1 immunity to Th2 type of immunity. However, in tuberculosis no clear cut transformatioin from TH1 to Th2 type of immune response with progression of disease has been noted as has been seen in leprosy or Johne's disease. Therfore this complex immunological facets have to be understood in a greater detail in future. Further, the mechanism of early events of host-pathogen interaction largely remains unknown. To unravel this, local immunity has to be understood at a greater detail. It is expected that using all the modern techniques in immunobiology a clear picture of the immunological net work against the pathogen would be brought out in the near future which might help in designing a suitable vaccine for protection against mycobacterial diseases.

REFERENCES

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. 1999. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global

Surviellance and Monitoring Project. JAMA 282:677-86.
2. Niemann S, Rusch-Gerdes S, Joloba ML, Whalen CC, Guwatudde D, et al.2002. *Mycobacterium africanum* subtype II is associated with two distinct genotypes and is a major cause of human tuberculosis in Kampala, Uganda. J Clin Microbiol 40:3398-405.
3. Tonjum T, Welty DB, Jantzen E, Small PL. 1998. Differentiation of Mycobacterium ulcerans, *M. marinum* an *M. haemophilum*: mapping of their relationships to *M. tuberculosis* by fatty acid profile analysis, DNA-DNA hybridization and 16SrRNA gene sequence analysis. J Clin Microbiol 36: 918-25.
4. Barker LP, George KM, Falkow S, Small PL. 1997. Differential trafficking of live and dead *Mycobacterium marinum* organisms in macrophages. Infect Immun 65: 1497-504.
5. Ramakrishnan L, Federspiel NA, Falkow S. 2000. Granuloma-specific expression of Mycobacterium virulence proteins from glycine-rich PE-PGRS family. Science 288:1436-39.
6. Solomon JM, Leung G, Isberg RR. 2003. Intracellular replication of *M.marinum* within D. discoideum: efficient replication in the absence of host coronin. Infect Immun 71:3578-86.
7. Talaat AM, Reimschuessel R, Wasserman SS, Trucksis M. 1998. Goldfish, Carassius auratus, a novel animal model for the study of *Mycobacterium marinum* pathogenesis. Infect Immun 66: 2938-42.
8. George KM, Chatterjee D, Gunawardana G, Welty D, Hayman J, et al. 199. Mycolactone: a polyketide toxin from Mycobacterium ulcerans required for virulence. Science 283:854-57.
9. Ridley DS, and Jopling WH. 1966. Classification of leprosy according to immunity; a five group system. Int J Lepr 34:255-73.
10. Cangelosi GA, Clark-Curtiss J, Behr MA, Bull T, Stinear T. 2003. Biology of pathogenic mycobacteria. In Pathogenic Mycobacteria in Water, ed. J. Bartram. Geneva: WHO and USEPA.
11. Inderlied CB, Kemper CA, Bermudez LE.1993. The Mycobacterium avium complex. Cli Microbiol Rev 6:266-310
12. Dannenberg AM+. 1989. Immune mechanisms in the pathogenesis of pulmonary tuberculosis. Rev Infect Dis 11(suppl.)2:S369-78.
13. Adams DO. 1976. The granulomatous inflammatory response. A review. Am J Pathol 84: 164-91.
14. Flynn JL, Chan J. 2001. Immunology of tuberculosis. Ann Rev Immunol 19: 93-129.
15. Desjardins M, Huber LA, Parton RG, Griffiths G. 1994. Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. J Cell Biol 124: 677-88.
16. Desjardns M. 1995. Biogenesis of phagolysosomes: the 'kiss and run' hypothesis. Trends Cell Biol 5:183-86.
17. Cohn Z. 1963. The fate of bacteria within phagocytic cells. I. The degradation of isotopically labeled bacteria by polymorphonuclear leucocytes and macrophages. J Exp Med 117: 27-42.
18. Armstrong J, D'Arcy Hart P. 1971. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations

on fusion of lysosomes with phagosomes. J Exp Med 134: 713-40.
19. D'Arcy Hart P, Armstrong J, Brown CA, Draper P. 1972. Ultrastructural study of the behaviour of macrophages toward parasitic mycobacteria. Infect Immun 5: 803-7.
20. Goren M, D'Arcy Hart P, Young MR, Armstrong JA. 1976. Prevention of phagosome-lysosome fusion in cultured macrophages by sulfatides of *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 73: 2510-14.
21. Middlebrook G, Coleman CM, Schaeffer WB. 1959. Sulfolipid from virulent tubercle bacilli. Proc Natl Acad Sci USA. 45: 1801-4.
22. Goren M, Brokel O, Roller P, Fales HM, Das BC. 1976. Sulfatides of Mycobacterium tuberculosis: the structure of the proincipalsulfatide (SL-1). Biochemistry 15: 2728.
23. Gordon A, D'Arcy Hart P, Young MR. 1980. Ammonia inhibits phagosome- lysosome fusion in macrophages. Nature 286: 79-81.
24. D'Arcy Hart P, Young MR, Jordan MM, Perkins WJ, Geisow MJ. 1983. Chemical inhibitors of phagosome-lysosome fusion in cultured macrophages also inhibit saltatory lysosomal movements. Acombined microscopic and computer study. J Exp Med 158: 477-92.
25. Reyrat J-M, Lopez-Ramirez G, Ofredo C, Gicquel B, Winter N. 1996. Urease activity does not contribute dramatically to persistence of *Mycobacterium bovis* bacillus Calmette-Guerin. Infect Immun 64: 3934-36.
26. Harth G, Clemens DL, Horwitz MA. 1994. Glutamine synthetase of Mycobacterium tuberculosis: extracellular release and characterization of its enzymatic activity. Proc Natl Acad Sci USA 91: 9342-46.
27. Harth G, Horwitz MA. 1999. Aninhibitor of exported Mycobactrium tuberculosis glutamine synthetase selectively blocks the growth of pathogenic mycobacteria in axenic culture and in human monocytes: extracellular proteins as potential novel drug targets. J Exp Med 189: 1425-35.
28. Crowle A, Dahl R, Ross E, May MH. 1991. E vidence that vesicles containing living, virulent *Mycobacterium tuberculosis* or *Mycobacterium avium* in cultured human macrophages are not acidic. Infect Immun 59: 1823-31.
29. Sturgill-Kaszyeki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Gluck SL, Heuser J, Russel DG. 1994. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton ATPase. Science 263: 678-81.
30. Xu S, Cooper A, Sturgill-Koszyeki S, van Heyningen T, Chatterjee D, Orme I, Allen P, Russell DG. 1994. Intracellular trafficking in Mycobacterium tuberculosis and *Mycobacterium avium*- infected macrophages . J Immunol 153: 2568-78.
31. Ferrari G, Langen H, Naito M, Pieters JA. 1999. Coat protein on phagosomes involved in the intracellular survival of mycobacteri.

Cell 97: 435-47.
32. Schullar S, Neefjes J, Otenhoff T, Thole J, Young D. 2001. Coronin is involved in uptake of *Mycobacterium bovis* BCG in human macrophages but not in phagosome maintenance. Cell Microbiol 3: 785-93.
33. Beatty WL, Rhoades ER, Hsu DK, Liu FT, Russell DG. 2002. Association of a macrophage galctosidase-binding protein with Mycobacterim-coating phagosomes. Cell Microbiol 4: 167-76.
34. Chan J, Fujiwara T, Brennan P, McNeil M, Turco SJ, Sibille JC, Snapper M, Aisen P, Bloom BR. 1989. Microbial glycolipids: possible virulence factors that scavange oxygen radicals. Proc Natl Acad Sci USA 86: 2453-57.
35. Chan J, Fan X-D, Hunter SW, Brennan PJ, Bloom BR. 1991.Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. Infect Immun 59: 1755-61.
36. MacMicking J, Xie Q-W, Nathan C. 1997. Nitric oxid and macrophage function. Annu Rev Immunol 15: 323-50.
37. Chan J, Flynn JL. 1999. Nitric oxide in Mycobacterium tuberculosis infection. In: Nitric Oxide and Infection.Ed. F. Fang, pp281-310. New York:Plenum.
38. Shiloh M, Nathan CF. 2002. Reactive nitrogen intermediates and the pathogenesis of Salmonella and mycobacteria. Curr Opin Microbiol 3: 35-42.
39. Nicholoson S, Bonecini-Almedia M, Siva JRL, Nathan C, Xie Q-W, Mumford R, Weidner JR, Calaycay J, Geng J, Boechat N, Linhares C, Rom W, Ho JL. 1996. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. J Exp Med 184: 2293-302.
40. Wang C-H, Liu C-Y, Lin H-C, Yu C-T, Chung KF, Kuo HP. 1998. Increased exhaled nitric oxide in active pulmonary tuberculosis due to inducible NO synthase upregulation in alveolar macrophages. Eur Respir J 11: 809-15.
41. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. 1998. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388: 394-97.
42. Medzhitov R, Preston-Hurlburt P, Koppe, Stadlen A, Chen C, Ghosh S, Jameway CA Jr. 1988. MyD88 is an adapter protin in the hToll/IL-1 receptor family signaling pathways. Moll Cell 2: 253-58.
43. Underhill DM, Ozinsky A, Smith KD, Aderem A. 1999. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Pro Natl Acad Sci USA 96:14459-63.
44. Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ. 1999. Human toll-like receptors mediate cellular activation of *Mycobacterium tuberculosis*. J Immunol 163: 3920-27.
45. Gehring AJ, Dobos KM, Belisle JT, Hardin CV, Boom WH. 2004. Mycobacterium tuberculosis LprG (Rv1411c): A novel TLR-2 ligand that inhibits human macrophages classII MHC antigen processing. J Immunol 173: 2660-68.

46. Ernest JD. 1998. Macrophage receptors for *Mycobacterium tuberculosis*. Infect Immun **66:** 1277-81.
47. Pieters J, Gatfield J. 2002. Hijacking the host survival of pathogenic mycobacteria inside macrophages. Trends Micrbiol **10:** 142-46.
48. Gatfield J, Pieters J. 2000. Essential role for cholesterol in entry of mycobacteria into macrophages. Science **288:** 1647-50.
49. Peyron P, Bordier C, N'Diaye EN, Maridonneau-Parini I. 2000. Nonopsonic phagocytosis of Mycobacterium kansasii by human neutrophils depends on cholesterol and is mediated by CR3 associated with glycosylphosphatidylinositol- anchord proteins. J Immunol **165:** 5186-91.
50. Dannenberg AM Jr. 1994. Immunopathogenesis of pulmonary tuberculosis. Hosp Pract **28:** 51-58.
51. Leemans JC, Juffermans NP, Florquin S, van Roajen N, Vervoodeldonk MJ et al. 2001. Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. J Immunol **166:** 4604-11.
52. Teitelbaum R, Schubert W, Gunther L, Kress Y, Macaluso F, et al. 1999. The M cell as aportal of entry to the lung for the bacterial pathogen- *Mycobacterium tuberculosis*. Immunity **10:** 641-50.
53. Davis JM, Clay H, Lews JL, Ghori N, Herbomel P, Ramakrishnan L. 2002. Real-time visualization of Mycobacterium-macrophage interactions leading to initiation of granuloma formation in zebrafish embryos. Immunity **17:** 693-702.
54. Peters W, Ernst ID.2003. Mechanisms of cell recruitment in the immune response to *Mycobacterium tuberculosis*. Microbes Infect **5:** 151-58.
55. Schnappinger D. et al. 2003. J Exp Med **198:** 693-704.
56. Adams DO. 1976. The granulomatous inflammatory response. A review. Am J Pathol **84:** 164-91.
57. Dannenberg AM Jr, Rook GA 1994. Pathogenesis of pulmonary tuberculosis: an interplay of tissue-damaging and macrophage-activating immune response-dual mechanisms that control bacillary multiplication. In: Tuberculosis Pathogenesis, Protection and Control. Ed. B.R.Bloom, Washington DC:ASM,pp. 459-83
58. Dannenberg AM Jr, Rook GA. 1994. Rabbit model of tuberculosis. In: Tuberculosis Pathogenesis, Protection and Control. Ed. B.R.Bloom, Washington DC: ASM, pp. 149-56.
59. McMurry DN. 1994. Guinea pigmodel of tuberculosis. In: Tuberculosis Pathogenesis, Protectioin and Control. Ed. B.R.Bloom, Washington DC: ASM,pp. 135-47.
60. Orme IM, Collins FM. 1994. Mouse model of tuberculosis Pathogenesis, Protection and Control. Ed. BR. Bloom, Washington DC: ASM, pp. 13-34.
61. Ladel CH, Szalay G, Reidel D, KaufmanSHE 1997. Interleukin-12 secretion by *Mycobacterium tuberculosis* infected macrophages. Infect Immun **65:** 1936-38.
62. Henderson RA, Watkins SC, Flynn JL. 1997. Activation of human

dendritic cells following infection with *Mycobacterium tuberculosis*. J Immunol **159:** 635-43.

63. Sano K, Handea K, Tamura G, Shirato K. 1999. Ovalbumin (OVA) and *Mycobacterium tuberculosis* bacilli a Th1-dominant phenotype and ameliorate murine tracheal eosinophilia. Am J Resp Cell Molec Biol **20:** 1260-67.

64. Cooper AM, Roberts AD, Rhoades ER, Callahan JE, Getzy DM, Orme IM. 1995. The role of interleukin-12 in acquired immunity to *Mycobacterium tuberculosis*. Infection. Immunology **84:** 423-32.

65. Cooper AM, Magram J, Ferrante J, Orme IM. 1997. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis*. J Exp Med **186:** 39-45.

66. Lowrie DB, Tascon RE, Bonato VLD, Lima VMF, Faccioli LH, Stavropoullos E, Colston MJ, Hewinson RG, Moelling K, Silva CL. 1999. Therapy of tuberculosis in mice by DNA vaccination. Nature **400:** 269-71.

67. Schluger NW, Rom WN.1998. The host immune response to tuberculosis. Am J Respir Crit Care Med **157:** 679-91.

68. Lalvani A, Brookes R, Wilkinson R, Malin A, Pathan A, Andersen P, Dockrell H, Pasvol G, Hill A. 1998. Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA **95:** 270-5.

69. Serbina NV, Flynn JL. 1999. Early emergence of CD8+ T cells primed for production of type 1 cytokines in the lungs of *Mycobacterium tuberculosis*-infected mice. Infect Immun **67:** 3980-88.

70. Orme IM, Roberts AD, Griffin JP, Abrams JS. 1993. Cytokine secretion by CD4 T lymphocytes acquired in response to *Mycobacterium tuberculosis*. J Immunol **151:** 518-25.

71. Chan J Xing Y, Magliozzo RS, Bloom BR. 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitogen intermediates produced by activated murine macrophages. J Exp Med **175:** 1111-22.

72. Denis M. 1991. Interferon-gamma-treated murine macrophages inhibit growth of tubercle bacilli via the generation of reactive nitrogen intermediates. Cell Immunol. **132:** 150-57.

73. Cooper AM, Dalton DK, Stewart TA. Griffin JP, Russell DG, Orme IM,.1993. Disseminated tuberculosis in IFN-γ gene disrupted mice. J Exp Med **178:** 2243-48.

74. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart T, Bloom BR. 1993. An essential role of interferon-γ in resistance to *Mycobacterium tuberculosis* infection. J Exp Med **178:** 2249-54.

75. Ottenhoff TH, Kumararatne D, Casanova JL. 1988. Novel human immunodeficiency reveal the essential role of type-1 cytokines in immunity to intracellular bacteria. Immunol Today **19:** 491-94.

76. Lin Y, Zhang M, Hofman FM, Gong J, Barnes PF. 1996. Absence

of a prominent TH2 cytokine response in human tuberculosis. Infect Immun 64: 1351-56.

77. Zhang M, Lin Y, Iyer DV, Gong J, Abrams JS, Barnes PF. 1995. T cell cytokine responses in human infection with *Mycobacterium tuberculosis*. Infect Immun 63: 3231-34.

78. Ting LM, Kim AC, Cattamanchi A, Ernst JD. 1999. *Mycobacterium tuberculosis* inhibits IFN-γ transcriptional responses without inhibiting activation of STAT1. J Immunol 163: 3898-906.

79. Bindu R, Hoshino Y, Kuwabara K, Belitskaya I, Prabhakar S, Canova A, Gold JA, Condos R, Pine RI, Brown S, Rom WN, Weiden MD. 2004. Aerosolized gamma interferon(IFN-γ) induces expression of the genes encoding the IFN-? inducible 10-kilodalton protein but not inducible nitric oxiide synthase in the lung during tuberculosis. Infect Immun 72: 1275-83.

80. Qiao Y, Prabhakar S, Coccia EM, Weiden M, Canova A, Giacomini E, Pine R. 2002. Host defence reponses to infection by *Mycobacterium tuberculosis*: induction of IRF-1 and a serine protease inhibitor. J Biol Chem 277: 22377.

81. Remoli MEE, Giacomini E, Lutfalla G, Dondi E' Orefici G, Battistini A, Uze G, Pellegrini S, Coccia EM. 2002. Selective expression of type 1 FN genes in human dendritic cells infected with *Mycobacterium tuberculosis*. J Immunol 169: 366.

82. Pine R. 2002. Review: IRF and tuberculosis. J Interferon Cytokine Res 22: 15.

83. Qiao Y, Prabhakar S Canova A, Hoshino Y, Weiden M, Pine R. 2004. Posttranscriptional inhibition of gene expression by *Mycobacterium tuberculosis* offests transcriptional synergism with IFN-? and posttrnascriptional upregulatrion by IFN-γ. J Immunol 172: 2935-43.

84. Cooper AM, Pearl JE, Brooks JV, Ehlers S, Orme IM. 2000. Expression of the nitric oxide synthase 2 gene is not essential for early control of *Mycobacterium tuberculosis* in the murine lung. Infect Immun 68: 6879-82.

85. Giacomini E, Lona E, Ferroni L, Miettinen M, Fattorini L, Orifici G, Julkunen I, Coccia EM.2001. Infection of human macropahges and dendritic cells with *Mycobacterium tuberculosis* induces a differential cytokine gene expression that modulates T cell response. J Immunol 166: 7033-41.

86. Weiden M, Tanaka N, Qiao Y, Zhao BY, Honda Y, Nakata K, Canova A, Levy DE, Rom WN, Pine R. Differentiation of monocyttes to macrophages switches the *Mycobacterium tuberculosis* effectn on HIV-1 replication from stimulation to inhibition: modulation of interferon response and CCAAT/ enhancer binding protein B expression. J Immunol 165: 2028-39.

87. Giosue S, Casarini M, Alemanno L, Galluccio G, Mattia P, Pedicelli G, Rebek L, Bisetti A, Ameglio F. 1998. Effects of aerosolized interferon-alpha in patients with pulmonary tuberculosis. Am J Respir Crit Care Med 158: 1156-62.

88. Prabhakar S, Qiao Y, Hoshino Y, Weiden M, Canova A, Giacomini

E, Coccia E, Pine R. 2003. Inhibition of response to alpha interferon by *Mycobacterium tuberculosis*. Infect Immun **71:** 2487-97.

89. Seah GT, Rook GAW. 2001. IL-4 influences apoptosis of mycobacterium -reactive lymphocytes in the presence of TNF-α. J Immunol 167: 1230-37.

90. Fenhalls G, Wong A, Bezuidenhout J, van Helden P, Bardin P, Luckey PT. 2000. In situ production of gamma interferon, interleukin-4, and tumor necrosis factor alpha mRNA in human lung tuberculosis granuloma. Infect Immun **68:** 2827-36.

91. Ladel CH, Szalay G, Reidel D, Kaufmann SHE. 1997. Interleukin-12 secretion by *Mycobacterium tuberculosis* infected macrophages. Infect Immun **65:** 1936-38.

92. Henderson RA, Watkins SC, Flynn JL. 1997. Activation of human dendritic cells following infection with *Mycobacterium tuberculosis*. J Immunol **159:** 635-43.

93. Serbina NV, Flynn JL. 1999. Early emergence of CD8+ T cells primed for production of type 1 cytokines in the lungs of *Mycobacterium tuberculosis*-infected mice. Infect Immun **67:** 3980-88.

94. Banes PF, Abrams JS, Lu S, Sieling PA, Rea TH, Modlin RL. 1993. Patterns of cytokine production by Mycobacterium-reactive human T-cell clones. Infect Immun **61:** 197-203.

95. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, Schreiber R, Mak TW, Bloom BR. 1995. Tumor necrosis factor-a is required in the protective immune reponse against *M. tuberculosis* in mice. Immunity **2:** 561-72.

96. Bean AGD, Roach DR, Briscoe H, France MP, Korner H, Sedgwick JD, Britton WJ. 1999. Structural deficiencies in granulomma formation in TNF-gene targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection which is not compensated for by lymphotoxin. J Immunol **162:** 3504-11.

97. Liew FY, Li Y, Millot S. 1990. Tumor necrosis factor -a synergizes with IFN-r in mediating killing of Leishmania major through the induction of nitric oxide. J Immunol **145:** 4306-10.

98. Ehler S, Benin J, Kutschn S, Endres R, Rietschel ET, Pfeffer K. 1999. Fatal granuloma necrosis without exaccerbated mycobacterial growth in tumor necrosis factor receptor p55 gene-deficient mice intravenously infected with *Mycobacterium avium*. Infect Immun **67:** 3571-79.

99. Kindler V, Sappino A-P, Grau GE, Piguet P-F, Vassalli P. 1989. The inducing role of tumor necrosis factor in the development of bacterial granulomas during BCG infection. Cell **56:** 731-40.

100. Gracia I, Miyazaki Y, Marchal G, Lesslauer W, Vassalli P. 1997. High sensitivity of transgenic mice expressing soluble TNFR 1 fusion protein to mycobacterial infections : synergistic action of TNF and IFN-gamma in the differentiation of protective granules. Eur J Immunol **27:** 3182-90.

101. Kindler, V, Sappino A-P, Grau GE, Piguet P-f, Vassalli, P. 1989. The

inducing role of tumor necrosis factor in the development of bacterial granules during BCG infection. Cell 56: 731-40.
102. Rook GAW. 1990. Mycobacteria, cytokines and antibiotics. Pathol Biol (Paris) 38: 276-80.
103. Rook GAW, Taverne J. Leveton C, Steele J. 1987. The role of gamma interferon, vitamin D3 metabolites and tumor necerosis factor in the pathogenesis of tuberculosis. Immunology 62: 229-34.
104. Rojas M, Oliver M, Gros P, Barrera LF, Gracia LF. 1999. TNF-a and IL-10 modulate the induction of apoptosis by virulent *Mycobacterium tuberculosis* in murine macrophages. J Immunol 162: 6122-31.
105. Murray PJ, Yang L, Onufryk C, Tepper RI, Young RA. 1997. T cell-derived IL-10 antagonizes macrophage function in mycobacteria infection. J Immunol 158: 315-21.
106. North RJ. 1998. Mice incapable of making IL-4 and IL-10 display normal resistance to infection with *Mycobacterium tuberculosis*. Clin Exp Immunol 113: 55-58.
107. Ladel CH, Blum C, Dreher A, Reifenberg K, Kopf M, Kaufman SHE. 1997. Lethal tuberculosis in interleukin-6-deficient mutant mice. Infect Immun 65:4843-49.
108. Saunders BM, Frank AA, Orme IM, Cooper AM. 2000. Interleukin-6 induces early inerferon production in the infected lung but is not required for generation of specific immunity to *Mycobacterium tuberculosis* infection. Infect Immun 68: 3322-26.
109. Hirsch CS, Ellner JJ, Blikhorn R, Toossi Z. 1997. In vitro restoration of Tcell responses in tuberculosis and augmentation of monocyte effector function against *Mycobacterium tuberculosis* by natural inhibitors of transforming growth factor Beta. Proc Natl Acad Sci USA 94: 3926-31.
110. Toosi Z, Gogato P, Shratsuchi H, Young T, Ellner JJ. 1995. Enhanced production of TGF-beta in tuberculosis granulomatous lung lesions. J Immunol 154: 465-
111. Dahl KE, Shiratsuchi H, Hamilton B, Ellner JJ, Toossi, Z. 1996. Selective induction of transforming growth factor ß in human monocytes by lipoarabinomannan of *Mycobacterium tuberculosis*. Infect Immun 64: 399-405.
112. Maeda J, Ueki N, Ohkawa T, Iwahashi N, Nakano T, Hada Y, Hagishino K. 1993. Local production and localization of transforming growth factor-ß in tuberculous pleurisy. Clin Rxp Immunol 92: 32-38.
113. Olobo JO, Geletu M, Demissie T, Eguale K, Hiwot K, Aderaye G, Britton S. 2001. Circulating TNF-α, TGF-ß and IL-10 in tubrculosis patients and healthy contacts. Scand J Immunol 53: 85-91.
114. Light RW, Cheng DS, Lee YC, Rogers J, Davidson J, Lane KB. 2000. A single intrapleural injection of transforming growth factor-ß(2) produces an excellent pleurodsis in rabbits. Am J Respir Crit Care Med 162: 98-104.

115. Dlugovitzky D, Bay ML, Rateni L, Urizar L, Rondelli CF, Largacha C, Farroni MA, Moteni O, Battasso OA. 1999. In vitro synthesis of interferon-a, interleukin-4, transforming growth factor ß and interleukin 1ß by peripheral blood mononuclear cells from tuberculosis patients: relationship with the severity of pulmonary involvement. Scand J Immunol 49: 210-17.

116. Espevik T, Nissen-Meyer J. 1986. A highly sensitive cell line, WEHI164 clone 13, for measuring cytotoxic factor/tumor necrosis factor for human monocytes. J Immuno methods 95:99-105.

117. Orme I, Collins FM. 1984. Adoptive protection of the *Mycobacterium tuberculosis*-infected lung: dissociation between cells that passively transfer protective immunity and those that transfer delayed hypersensitivity. Cell Immunol 84: 113-20.

118. Muller I, Cobbold S, Waldmann H, Kaufman SHE. 1987. Impaired resistance to *Mycobacterium tuberculosis* infection after selective in vivo depletion of L3T4+ and Lyt2+ T cells. Infect Immunol 55: 2037-41.

119. Orme I, Collins F. 1983. Protection against *Mycobacterium tuberculosis* by adoptive immunotherapy. J Exp Med 158: 74-83.

120. Tascon RE, Stavropoulos E, Luckacks KV, Colston MJ. 1998. Protection against *Mycobacterium tuberculosis* infection by CD8 T cells requires production of Gamma interfron. Infect Immun 66: 830-34.

121. Caruso AM, Serbina N, Klein E, Triebold K, Bloom BR, Flynn JL. 1999. Mice deficient in CD4 T cells have only transiently diminished levels of IFN-?, yet succumbs to tuberculosis. J Immunol 162: 5407-16.

122. Barnes PF, Bloch AB, Davidson PT, Snider D. 1991. Tuberculosis in atients with human immunodeficiency virus-infection. N Eng J Med 324: 1644-50.

123. Lucas S, Nelson AM. 1994. Pathogenesis of tuberculosis in human immuno-deficiency virus-infected people. In: Tuberculosis pathogenesis, protection and control. Ed. BR Bloom. ASM Press, pp.503-13.

124. Barnes PF, Fong F-J, Brennan PJ, Twomey PE, Mazumdar A, Modlin RL. 1990 Local production of TNFα and IFN γ in tuberculous pleuritis. J Immunol. 45: 149-54.

125. Silver RF, Li Q, Boom HW, , Ellner JJ. 1998. Lymphocyte dependent inhibition of growth of virulent *Mycobacterium tuberculosis* H37Rv within human monocytes: Requiremnet for CD4+ T cells in purified protein dervative-positive, but not in purified protein derivetive-negative subjects. J Immunol 160: 2408-17.

126. Scanga CA, Mohan VP, Yu K, Joseph H, Tanaka K, Chan J, Flynn JL. 2000. Depletion of CD4+ T cells causes reactivation of murine persistent tuberculosis Despite continued expression of IFN-γ and NOS2. J Exp Med 192: 347-58.

127. Stenger S, Niazi KR, Modlin RL. 1998. Down-regulation of CDI on antigen presenting cells by infection with *Mycobacterium tuberculosis*. J Immunol 161:3582-88.

128. Flynn JL,Goldstein MM, Triebold KJ, Bloom BR.1992. Major histocompatibility complex class 1-restricted T cells are required in resistance to *Mycobacterium tuberculosis* infection. Proc Natl Acad Sci USA 89: 12013-17.

129. Behar SM, Dascher CC, Grusby MJ, Wang CR, Brenner PB. 1999. Susceptibility of mice deficient in CD1D or TAP1 to infection with *Mycobacterium tuberculosis*. J Exp Med 189: 1973-80.

130. Sousa AO, Mazzaccaro R, Russell DG, Lee FK, Turner OC, Hong S, Van Kaer L Bloom BR. 1999. Relative contributions of distinct MHC class I-dependent cell populations in protection to tuberculosis infection in mice. Proc Natl Acad Sci USA 97: 4204-8.

131. Feng CG, Beam AGD, Hooi H, Briscoe H, Britton WJ. 1999. Increase in gamma interferon-secreting CD8+, as well as CD4+ T cells in lungs following aerosol infection with *Mycobacterium tuberculosis*. Infect Immun 67: 3242-47.

132. Zhu X, Stauss HJ, Ivanyi J, Vordermeier HM.1997. Specificity of CD8+ T cells from subunit vaccinated or infected H-2b mice recognising the 38kDa antigen of *Mycobacterium tuberculosis*. Int Immunol 9: 1669.

133. Silva CL, Lowrie DB.2000.Identification and charcterization of murine cytotoxic T cells that kill *Mycobacterium tuberculosis*. Infect Immun 68: 3269-74.

134. Mohagheghapour N, Gammon D, Kawamura LM, van Vollenhoven A, Benike CJ, Engleman EG. 1998. CTL response to *Mycobacterium tuberculosis*: Identification of an immunogenic epitope in the 19kDa lipoprotein. J Immunol 161: 2400-2403

135. Mcdonough KA, Kress Y, Bloom BR. 1993. Pathogenesis of tuberculosis: Interaction of *Mycobacterium tuberculosis* with macrophages. Infect Immun 61: 2763-73.

136. Serbina NV, Liu C-C, Scanga CA, Flynn JL. 2000. CD8+ Cytotoxic T lymphocytes from lungs of tuberculosis infected mice express perforin in vivo and lyse infected macrophages. J Immunol 165: 353-63.

137. Mazzaccaro RJ, Gedde M, Jensen ER, van Santem HM, Ploegh HL, Rock KL, Bloom BR. 1996. Major histocompatibility class I presentation of soluble antigen facilitated by *Mycobacterium tuberculosis* infection. Natl Acad Sci USA 93:11786-91.

138. Teitelbaum R, Cammer M, Maitland ML, Freitag NE, Condeelis R, Bloom BR. 1999. Mycobacterial infection in macrophages results in membrane permeable phagosomes. Proc Natl Acad Sci USA 96: 15190-95.

139. Porcelli SA, Modlin RL 1999. The CD1 system: antigen presenting molecules for T cell recognition of lipids and glycolipids. Ann Rev Immunol 17: 297-329.

140. Sieling PA, Ochoa M-T, Jullien D, Leslie DS, Sabet S, Rosat J-P, Burdick AE, Rea TH, Brenner MB, Porcelli SA, Modlin RL. 2000. Evidence for human CD4+ T cells in the CD1-restricted repertoire: derivation of mycobacteria -reactive T Cells from leprosy lkesions.

J Immunol 164: 4790-96.
141. Beckman EM, Porcelli SA, Morila CT, Behar SM, Furlong ST, Brenner MB. 1994. Recognition of a lipid antigen by CD1-restricted αß + T cells. Nature 372: 691-94.
142. Sieling PA, Chatterjee D, Porcelli SA, Prigozy TI, Mazzaccaro RJ, Soriano T, Bloom BR, Brenner MB, Kronenberg M, Brennan PJ, Modlin RL. 1995. CD1-restricted T cell recognition of microbial lipoglycan antigens. Science 269:3227-30.
143. Moody DB, Reinhold BB, Guy MR, Beckman EM, Frederique DE, Furlong ST, Ye S, Reinhold VN, Sieling PA, Modlin RL, Bersa GS, Porcelli SA. 1997. Structural requirements for glycolipid antigen recognition by CD1b-restricted T clls. Science 278: 283-86.
144. Moody DB, Urlichs T, Muhlecker W, Young DC, Gurcha SS, Grant E, Rosaat J-P, Brenner MB, Costello CE, Bersa GS, Porcelli SA. 2000. CD1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* Infection. Nature 404: 884-88.
145. 145. Beatty WL, Rhoades ER, Ulrich H-J, Chatterjee D, Heuser JE, Russell DG. 2000. Trafficking and release of mycobacterial lipids from infected macrophages. Traffic 1: 235-47.
146. Porcelli, A, Morita CT, Brenner MB. 1992. CD1b restricts the response of human CD4-8- T lymphocytes to a microbial antigen. Nature 360: 593-97.
147. Sieling PA, Jullien D, Dahlem M, Rea TH, Modlin RL, Porcelli SA. 1999. CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. J Immunol 162: 1851-58.
148. Stenger S, Mazzaccaro R, Uymura K, Cho S, Barnes P, Rosat J, Sette A, Brenner M, Porcelli S, Bloom B, Modlin RL. 1997. Differential effects of cytolytic T cell Subsets on intracellular infection. Science 276: 1684-87.
149. Stenger S, Hansen DA, Teitelbaum R, Dewan P, R NK, Froelich CS, Ganz T, Thomauszynski S, Melian A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL. 1998. An antimicrobial activity of cytotoxic T cells mediated by Granulysin. Science 282: 121-25.
150. Boom WH. 1999. Gammadelta T cells and *Mycobacterium tuberculosis*. Microbes Infect 1: 187-95.
151. Schild H, Mavadat N, Litzenberger C, Ehrich EW, Davis MM, Bluestone J, Matis L, Draper RK, Chein YH. 1994. The nature of major histocompatibility Complex recognition by gamma delta T cells. Cell 76: 29-37.
152. Dieli F, Ivanyi J, Marsh P, Williams A, Naylor I, Sireci G, Caccamo N, Sano DC, Salerno A. 2003. Characterization of lung gamma delta T cells following Intranasal infection with *Mycobacterium bovis* bacillus Calmette- Guerin. J Immunol 170: 463-69.
153. Inoue T, Yoshikai Y, Matsuzaki G, Nomato K. 1991. Early appearing gamma/delta- bearing T cells during infection with Calmette Guerin bacillus. J Immunol 146: 2754-62.
154. Landel CH, Blum C, Dreher A, Reifenberg K, Kaufman SH 1995. Protective role of gamma/delta T cells and alpha/beta T cells in

tuberculosis. J Immunol 25: 2877-81.
155. Havlir DV, Ellner JJ, Chervenak KA, Boom WH. 1999. Selective expansion of human gamma delta T cells by monocytes infected with live Mycobacterium tuberculosis. J Clin Investig 87: 729-33.
156. Kabelitz D, Bender A, Schondelmaier S, Schoel B, Kaufman SH. 1990. A large fraction of human peripheral blood gamma/delta+ T cells is activated by *Mycobacterium tuberculosis* but not by its 65kD heat shock protein. J Exp Med 171: 667-69.
157. Tsukaguchi K, Balaji KN, Boom WH.1995. CD4+ alpha beta T-cell and gamma/delta T-cell responses to *Mycobacterium tuberculosis*. Similarities and differences in Ag recognition, cytotoxic effector function, and cytokine production. J Immunol 154: 1786-96.
158. Cosivi O, Grange JM, Daborn CJ, Raviglione MC, Fujikura T, Cousins D, Robinson RA, Huchzermeyer HF, de Kantior I., Meslin FX 1998. Zoonotic Tuberculosis due to *Mycobacterium bovis* in developing countries. Emerg Infect Dis 4: 59-70.
159. Niell SD, Cassidy J, Hanna J, Mackie DP, Pollock JM, Clements A, Walton E, Bryson DG, 1994. Detection of *Mycobacterium bovis* infection in skin-test Negative cattle with an assay for bovine interferon-gamma. Vet Rec 135: 134-35.
160. Rhodes SG, Buddle BM, Hewinson RG, Vordermeier M. 2000. Bovine tuberculosis: immune responses in the peripheral blood and at the site of active disease. Immunology 99: 195-202.
161. Wood PR, Corner LA, Rothel JS, Baldock C, Jones SL, Cousins DB, McCormick BS, Francis BR, Creeper J, Tweddle NE.1991. Field comparison of the interferon-gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. Aust Vet J 68: 286-90.
162. Wood PR, Corner LA, Plackett P. 1990. Development of a simple rapid in vitro cellular assay for bovine tuberculosis based on the production of interferon-gamma. Res Vet Sci 49: 46-49.
163. Rhodes SG, Palmer N, Graham P, Bianco AE, Hewinson RG, Vordermeier HM 2000. Distinct response kinetics of gamma interferon and interleukin-4 in bovine tuberculosis. Infect Immun 68: 5393-400.
164. Ott SL, Wells SJ, Wagner BA 1999. Herd-level economic losses associated with Johne's disease on US dairy operations. Prev Vet Med 40: 179-92.
165. Johnson-Ifearulundu Y, Kaneene JB, Lloyd JW. 1999. Herd-level economic analysis of the impact of paratuberculosis on dairy herds. J Am Vet Med Assoc 214: 822-25.
166. Cocilo C, Gilot P, Coene M, de Kesel M, Poupart P, Vannuffel P. 1994. Paratuberculosis. Clin Microbiol Rev 7:328-45.
167. Momotani E, Whipple DL, Thiermann AB, Cheville NF. 1988. Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Vet Pathol 25: 131-37.
168. Siguroardottir OG, Press GM, Evensen O. 2001. Uptake of

Mycobacterium avium subsp. *paratubberculosis* through the distal small intestinal mucosa in goats: an ultrastructural study. Vet Pathol 38: 184-89.

169. Tizard I. 2000. Veterinary immunology: an introduction. Sixth Ed. WB Saunders, Philadelphia, Pa.

170. Ratliff TL, McCarthy R, Telle WB, Brown EJ. 1993. Purification of a mycobacterial adhesin for fibronectin. Infect Immun 61: 1889-94.

171. Schorey JS, Holsti MA, Ratliff TL, Allen PM, Brown EJ. 1996. Characterization fibronectin-attachment protein of *Mycobacterium avium* reveals a fibronectin binding motif conserved among mycobacteria. Mol Microbiol 21: 321-29.

172. Schorey JS, Li Q, McCourt Dw, Bong-Mastwek M, Clark-Curtis JE. Ratliff TL, Brown EJ. 1995. A *Mycobacterium leprae* gene encoding a fibronectin binding protein is used for efficient invasion of epithelial cells and Schwann cells. Infect Immun 63: 2652-57.

173. Secott TE, Lin TL, Wu CC. 2001.Fibronectin attachment protein homologue mediates fibronectin binding by *Mycobacterium avium* subsp. *paratuberculosis*. Infect Immun 69: 2075-82.

174. Zhao W, Schrrey JS, Groger R, Allen PM, Brown EJ, Ratliff TL. 1999. Characterization of the fibronectin binding motif for a unique mycobacterial Fibronectin attachment protein. J Biol Chem 274: 4521-26.

175. Kuroda E, Brown EJ, Telle WB, Russell DG, Ratliff, TL. 1993. Characterization and internalization of bacillus-Calmette-Guerin by human bladder tumor cells. J Clin Investig 91: 69-76.

176. Scott TE, Lin TL, Wu CC. 2002. Fibronectin attachment protein is necessary for efficient attachment and invasion of epithelial cells by *Mycobacterium avium* subsp. *paratuberculosis*. Infect Immun 70: 2670-75.

177. Clark MA, Hirst BH, Jepson MA 1998. M-cell surface beta 1 integrin expression and invasion imediated targetting of Yersinia pseudotuberculosis to mouse Peyer's patch M cells. Infect Immun 66: 1237-43.

178. Secott TE, Lin TL, Wu CC. 2004. *Mycobacterium avium* subsp. *Paratuberculosis* fibronectin attachment protein facilitates M-cell targetting and invasion through a fibronectin bridge with host integrins. Infect Immun. 72: 3724-32.

179. Whitlock RH, Buergelt C 1996. Preclinical and clinical manifestations of paratuberculosis (including pathology). Vet Clin North Am Food Anim Pract 12: 345-46.

180. Burrells C, Clarke CJ, Colston A, Kay JM, Porter J, Little D, Sharp JM. 1998. A study of immunological responses of sheep clinically affected with pararatuberculosis (Johne"s disease). The relationship of blood mesenteric lymh node and intestinal lymphocyte responses to gross and microscopic pathology. Vet Immunol Immunopathol 66: 343-58.

181. Strohmeier GR, Fenton MJ. 1999. Roles of lipoarabinomannan in

the pathogenesis of tuberculosis. Microbes Infect 1: 709-17.
182. Navarro JA, Ramis G, Seva J, Pallares FJ, Sanchez J. 1998. Changes in lymphocyte subsets in the intestine and mesenteric lymph nodes in caprine paratuberculosis. J Comp Pathol. 118: 109-21.
183. Billman-Jacob H, Carrigan M, Cockram F, Corner A, Gill IJ, Hill JF, Jessep T, Millner AR, Wood PR. 1992. A comparison of the interferon gamma assay with the absorbrd ELISA for the diagnosis of Johne's disease in cattle. Aust Health Vet J 69: 25-28.
184. Coussens PM. 2000. Interaction between *Mycobacterium paratuberculosis* and the bovine immune system. Aim Health Rev 2: 141-61.
185. Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP, Jacobson RH, Stabel JR. 2001. Evaluation of commercial ELISA for diagnosis of paratuberculosis in cattle. J AM Vet Med Assoc 218: 1163-66.
186. Stabel JR. 2000. Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet Microbiol 77: 465-73.
187. Coussens PM, Verman N, Coussens MA, Elftman MD, McNulty AM. 2004. Cytokine gene expression in peripheral blood mononuclear cells and tissue in cattle infected with *Mycobacterium avium* subsp. *paratuberculosis*: evidence for an inherent proinflammatory gene expression pattern. Infect Immun 72: 1409-22.
188. Sengupta U. 1993. Cell mediated immunity-An update. Int J Lepr 61: 439-189.
189. Narayana RB, Girdhar BK, Sengupta U, Desikan KV. 1985. In vitro studies on dermal granulomas of human leprosy: cellular characteristics. Int J Lepr 53: 39
190. ModlinRL, Hofman FM, Taylor CR, Rea TH. 1982. *In situ* charcterization of T lymphocyte subsets in leprosy granulomas (letter). Int J Lepr 50: 361-62.
191. Sengupta U. Immunopathology of leprosy; a state of the art. 2001. Int J. Lepr 69: S36-S41.
192. Modlin RL, Melancon-Kaplan J, Young SMM, Pirmez C, Kino H, Convit J, Rea TH, Bloom BR. 1988. Learning from lesions: pattern of tissue inflammation In leprosy. Proc Nat Acad Sci USA. 85: 1213-17.
193. Naryana RB, Bhutani LK, Sharma AK, Nath I. 1983. T-cell subsets in leprosy lesions in situ characterization using monoclonal antibodies. Clin Exp Immunol 51: 421-29.
194. Wakllach D, Flageul B, Bach M-A, Cottenot F. 1984. The cellular content of dermal leprous granulomas: an immunohistological approach. Int J Med 52: 318-26.
195. Mosmann TR, Coffaman RL. 1989. Th1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann Rev. Immunol 7: 145-73.
196. Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T-cell clones. Science 234: 279-82.

197. Mutis T, Kraakman EM, Cornelisse Ye, Haanen JBG, Spits H, De Vries RRP, Ottenhoff THM. 1993. Analysis of cytokine production by Mycobacterium reactive T-cells. Failure to explain *Mycobacterium leprae*-specific onresponsiveness of peripheral blood T-cells from lepromatous leprosy patients. J Immunol **105:** 4641-51.

198. Verhagen CE, Wierenga EA, Buffing AAM, Chand MA, Faber WR, Das PK. Reversal reaction in borderline leprosy is associated with a polarized shift to Trype 1-like *Mycobacterium leprae* T-cell reactivity in lesional skin: a follow-up Study. J Immunol **159:** 4474-83.

199. Mitra DK, DeRosa SC, Luke A, Balamurugan A, Khaitan BK, Tung J, Mehra NK, Tera AI, O'Garra A, Herzenberg LA, Roederer M. 1999. Differential Representation of memory T-cell subsets are characteristic of polarized immunity In leprosy and atopic diseases. Int Immunol **11:** 1801-10.

200. Modlin RL, Pirmez C, Hofman FM, Torigian V, Uyemura K, Rea TH, Bloom BR, Brenner MB 1989. T-cell receptors in human infectious disease lesions. Nature **339:** 544-48.