Review of Literature

The written records of measles can be traced back to the original writings of a Persian physician, Rhazes, who was also known as Abu Beer during the tenth century (Prebluad S. and Katz S., 1986). This disease was apparently known since the seventh century to the Hebrew physician Al Yehudi. Abu Beer referred to measles as 'hasbah', which, in Arabic, means eruption (Black F.L., 1982). The word 'measles' might have been derived from 'mesels', the Anglicized form of 'misellus', which in turn is a diminutive of the Latin word 'miser', meaning miserable and referring to the sufferer with various eruptions or sores. The presence of non-specific leprous sores was incorrectly identified with the disease called Morbilli. Thus measles came to be equated with the disease and not the sufferer of ill defined skin lesions.

It was Rhazes who appears to have been the first to make the distinction between measles and small pox (Wilson G.S., 1964). He considered measles to be a very severe disease 'more to be dreaded than small pox'. Although Rhazes did distinguish between the two diseases, he and others still probably considered them to be closely related. Furthermore, while he was aware of the seasonal nature of measles, he did not think the disease was infectious.

As observed by Wilson, the difference between measles and small pox was becoming closer by the beginning of the 17th century when annual bills of mortality in London in 1629 listed the two diseases separately (Wilson, 1964). Thomas Sydenham clearly described the clinical characteristics of measles during this time period and believed the disease to be infectious. It was, however, Francis Home, Scottish physician who worked in Edinburgh in the mid 18th century, who truly recognized the infectious nature of the illness in his attempts to prevent it. In 1758, he utilized an approach similar to variolization i.e. scarification technique to induce mild small
pox prior to the Jennerian vaccination. The absence of vesicular and pustular lesions, such as those seen in small pox and from which the variolation material was obtained, presented a challenge to Home. Antedating the knowledge of viremia preceding the rash of measles, he chose to utilize the blood from patients at the peak of their fever and the onset of the rash. Following such a technique, he inoculated twelve children with material from the blood of measles patient. Ten of them developed the rash that was typical of the disease, preceded by symptoms of upper respiratory infection. This technique came to be known as "morbillization", but was never adapted.

The information about epidemiology of measles was greatly increased by the classic investigation of measles epidemic on the Faroe Islands in 1846 by the young Danish physician, Peter Panum. He not only confirmed that measles was contagious, but also defined the 14-day interval between exposure and appearance of exanthem and observed that infection provided lifelong immunity (Stephen R., Preblud, Samuel L., Katz M.D., 1986).

In the year 1911, using infected material from acute cases, Goldberger and Anderson transmitted human measles infection to monkeys clearly demonstrating the existence of an infectious agent or a substance responsible for the disease. In 1954, Enders and Peebles successfully isolated the measles virus in human and monkey kidney tissue culture (Enders and Peebles, 1954). Adaptation of the virus to chicken embryo tissue culture paved the road to vaccine development. The vaccine was licensed in 1963.

**BIOLOGY OF MEASLES**

Three major developments have created more interest on the nature of measles virus than has been shown since the virus was first isolated by Enders and Peebles in 1954 and
then adapted to make a successful vaccine (Katz & Enders, 1959; Katz et al, 1962; Katz, 1965).

The most powerful stimulus was the realization that measles virus antigen was implicated in subacute sclerosing pan-encephalitis (SSPE), rare but lethal disease of man, which was followed by the isolation of measles like virus from infected brain (Connolly, 1972). The reasons why a very small proportion of children, previously infected with measles, should develop a fatal chronic measles infection of the brain, years later, remain unknown and will be solved only by studying the biology of measles virus in relation to immunological and other factors in the patient. Generally SSPE develops in immunologically noncompromized children 5 to 10 years after acute measles and usually leads to death within a month to year (ter Meulen et al, 1983). Measles inclusion body encephalitis (MIBE), a second type of disorder associated with measles, in turn has a shorter latent period and is found in immunosuppressed patients (Roos et al, 1981; Johnson, 1982), but its virological manifestations are similar to those of SSPE. It is characteristic for SSPE and MIBE that, although the infection is spread over essentially the whole brain, neither lytic virus nor inactive particles can be detected. It has been proposed that the lack of viral budding is specifically due to the absence or defect of the matrix (M) protein, which plays a key role in virus assembly (Hall et al, 1979). This suggestion was based on the failure to find antibodies against M protein in both the cerebrospinal fluid and serum of SSPE patients as well as on the inability to detect M protein in SSPE brain autopsy material (Hall & Choppin, 1981). Cloning and sequencing of the M genes from different SSPE and MIBE isolates revealed, in three cases, premature stop codons and in three other cases, biased hypermutation events which shifted the start and stop codons and changed dozens of amino acids in the M protein (Cattaneo R., Schmid, 1988). In four cases, however, analysis of the gene sequence revealed only several missense mutations causing no alterations of the reading frame and it
was, therefore, not clear whether these genes were able to produce functional M protein (Sheppard et al, 1986). A system was described by Isidro Ballart (June 1991) which allows one to assay the functionality of measles virus M genes of patients with SSPE via their cloned C' DNAs.

The second development was unexpected. Killed measles vaccine, which was considered safer than live attenuated vaccine by many workers, not only failed to give complete protection against measles, but also sensitized a certain number of those vaccinated, in such a way, that the subsequent natural infection caused aberrant and sometimes serious symptoms (Linnenman, 1973; Cherry J.D., 1972). This was enough to have stopped the use of killed measles virus with adjuvants as vaccines.

The third development is part of a new understanding that another chronic nervous disease, multiple sclerosis, has many features that suggest immunological disarrangements as a cause or consequence of the illness (Fraiser K.B. & Martin J., 1978).

**STRUCTURE AND COMPONENTS OF MEASLES VIRUS**

The measles virus is a spherical, single stranded RNA virus with a diameter of 120 - 250 nm. It is a member of the genus Morbillivirus, in the family Paramyxoviridae and is closely related to the canine distemper and rinderpest virus (Morgan E.M., Gershon A.A., Krugman S., Imagawa D.T.).

The central core contains helices and there are radially distributed projections on the outer membrane. The virus consists of an outer membrane covered with spikes, 9-15 nm in length, surrounding the tightly coiled helical nucleocapsid which has a diameter of 16 - 17 nm (Norrby E.).
1. PHYSICAL PROPERTIES

The buoyant density of measles virus has been determined by equilibrium sedimentation on CsCl or potassium tartrate gradients. The reported values lie in the range 1.224 to 1.240 g/cm$^3$. (Norrby, 1964; Numazaki and Karzon, 1966; Hall and Martin, 1973; Phillips and Bussell 1973). Studies by Rima have shown that the density of measles virus on Metrizamide is 1.19 g/cm$^3$. The variations in the density reported from various laboratories may be explained by the type of virus growth which is taking place. Chiarini and Norrby (1970) found that the density of measles virus in CsCl gradient was greatly influenced by the multiplicity of infection and passage history of the virus inocula. Oddo et al introduced the term UP virus (undiluted passage) and DP virus (diluted passage) and showed that the buoyant density of UP particles was 1.20 to 1.21 g/cm$^3$, whereas DP had a density of 1.23 to 1.25 g/cm$^3$ (Underwood and Brown, 1974). Jonston, Rima and Martin (unpublished results) have also found that measles virus purified on potassium tartrate gradients contained two components when the virus was passed undiluted, whereas only the heavier 1.23 g/cm$^3$ component was present when virus was grown after dilution of inoculum to 1:1000.

Philippe Calain and Laurent Roux (1988) published a comprehensive work on generation of defective interfering (DI) particles in the process of attenuation of various strains. Experimentally, successive undiluted passages of virus stock favour the generation of DI particles. DI particles, by interfering with their standard counterpart, reduce the yield of fully infectious virions and can lead to persistent infections in tissue cultures.

It is indicated from the findings of Hall et al that, nucleocapsid of only about one tenth of the intact genome can be incorporated into enveloped structures, although the contribution of RNA to the density of enveloped particles is so
small that this factor alone is not likely to be a major determinant of those differences.

2. NUCLEOCAPSID

Nucleocapsid of measles virus contains the intact virus genome and is responsible for part of the complement fixing (CF) activity of the virus particles. Nucleocapsid can be released from purified virus and isolated on sucrose or CsCl gradients (Fraiser & Martin, 1978). Electron microscopy shows that the helix from infected cells have characteristic hearing bone appearance with a diameter of 17 nm to 20 nm and the hollow core, when viewed along the short axis of structure, measures 7 nm. The buoyant density of nucleocapsid determined on CsCl gradient is 1.3 to 1.31 g/cm³ (Hall & Martin, 1973; Norrby & Hammerskyold, 1972). Sedimentation coefficients of between 265-280 S have been reported. Ultraviolet spectrum analysis and direct chemical studies (Hall and Martin, 1973; Waters & Bussel, 1974) have shown that the nucleocapsid of measles virus has an RNA content of 5 S, the remainder being composed of a single species of a protein of molecular weight approximately 60,000. Busell et al showed that the method of isolation of nucleocapsids from cells had an important bearing on the morphology of the particles and size of the protein. They isolated nucleocapsids from infected cells by use of proteolytic enzyme or simply by treatment of cells with EDTA and freezing and thawing.

When proteolytic enzymes were employed the nucleocapsids were rigid and tightly coiled helices, whereas if isolation was carried out in the absence of proteolytic enzyme, they had a more loosely coiled and flexible appearance. These observations are consistent with the morphology seen by the other workers, who have isolated nucleocapsids from purified virions. Furthermore, they showed that the protein isolated from nucleocapsids prepared without proteolytic enzymes had a molecular weight of 60,000 as a virion material (Hall and
Martin, 1973), however, when cells were harvested with trypsin, two proteins of molecular weight 39,000 and 24,000 were obtained (Rima & Martin).

The size of the nucleocapsids from infected cells have been studied by electron microscopy and sedimentation on sucrose gradients. Kiley et al demonstrated that the nucleocapsids isolated from measles infected cells were heterogeneous in length, although the ratio of RNA to protein remained the same. They also demonstrated that the nature of nucleocapsids present was related to passage history of the virus inocula. Undiluted virus inocula produced a mixture of 200 S and 110 S nucleocapsids whereas diluted virus gave predominantly 200 S species. Furthermore, isolation of RNA from the purified nucleocapsids fractions yielded RNA directly related to the length of nucleocapsid fragment and the 110 S component obtained RNA which sediments at 18 S and was only one tenth that of intact genome (Carter, 1973).

3. VIRION RNA

The first report of the size of the measles virion RNA was made by Schleuderberg (1971), who found that the sedimentation coefficient was 52.2 S relative to 50 S RNA of SV 5. Hall and Martin showed that the RNA sedimented at approximately 52 S was sensitive to ribonuclease. In all the preparations of virus examined, a second component sedimenting at 4 S was found, but this low molecular weight RNA was never found in purified nucleocapsids and was probably of cellular origin. Reports from numerous laboratories have indicated a range of sedimentation values for RNA from paramyxoviruses in the range of 50 to 57 S (Blair & Robinson, 1968; Compans & Choppin, 1968; Winston, 1973). The RNA of measles virus shows considerable similarities to that of other well characterized paramyxoviruses. The infectious genome is an intact single strand with a molecular weight of about 6.4 millions. The RNA is encapsidated by approximately 2000 protein subunits. M.J. Taylor (1991) has published an excellent
article on 'Identification of several different lineages of measles virus'. Measles virus is a monotypic virus which has been considered extra-ordinarily stable in terms of its serology and immune responses to it. However, analysis of the ability of different strains of the virus to bind monoclonal antibodies has revealed a low level of variability in the major antigens (Sheshberadaran et al, 1983) and more recently, strain variations have been studied directly by analysis of the nucleotide sequences of MV strains (Cattaneo et al, 1989). Most data is available for the matrix protein (M) gene of measles virus where a number of lytic growing strains (Bellini et al, 1986; Wong et al, 1987; Curran and Rima, 1988) and subacute sclerosing panencephalitis (SSPE) derived strains (compiled in Cattaneo et al 1989; Enami et al, 1989) have been analyzed. This gene has been studied in detail because it has been noted that, it, in particular, is defectively expressed in SSPE. Further analyses of the nucleocapsid protein (N), phosphoprotein (P), fusion protein (F) and haemagglutinin (H) have been published in the last two years, almost always comparing the only sequence known, in its totality (i.e. that of the Edmonston strain as passaged in various laboratories), with those of SSPE derived strains or measles virus derived from measles inclusion body encephalitis (MIBE). There have been many indications that the Edmonston strain is rather unrepresentative and that this comparison may give a false impression of the true rates of change between lytic measles virus and those sequences from SSPE derived strains.

The sequences of region of nucleocapsid protein gene, between nucleotides 1231 and 1686, encoding the C terminal 151 amino acid residues of the nucleocapsid protein have been determined for sixteen strains of measles virus (Taylor, 1991). The analysis of this showed that, it is highly divergent (upto 7.2 % divergence in the nucleotide sequence and 10.6 % divergence in the amino acid sequence between most distant strains) and that several lineages of measles virus can be found to co-circulate at a given time.
4. STRUCTURAL POLYPEPTIDE

Until 1973, no reports on the polypeptide composition of measles virus were available. Reports by Hall and Martin, and Walters and Bussell showed that the polypeptide composition was similar to other paramyxoviruses. Both studies involved the separation of $^{35}$S methionine or $^{14}$C amino acid labeled protein isolated from purified viruses by electrophoresis on SDS polyacrylamide gels. These initial reports indicated the presence of at least 6 polypeptides in the molecular weight range 80,000 to 35,000. There was also evidence of high molecular weight material which did not enter 7.5 % gels, especially when virus was not degraded exhaustively with mercaptoethanol. Bussell et al have also reported that a high molecular weight polypeptide of approximately 1,85,000 daltons is present and is the major component when virus particles are dissociated in the absence of a reducing agent. Their results suggest that this component is a major virus protein containing two polypeptides which are linked by disulphide bonds as reduction results in the appearance of similar polypeptides which co-migrate with P1 and P2.

There are six structural proteins. The haemagglutinins (H), the fusion (F) and the membrane (M) proteins form a part of the viral envelope, whereas the nucleocapsid (N), the phospho (P) and the large (L) proteins together with one viral RNA, represent the replication complex (Norrby, 1990). As documented in clinical and experimental studies, H and F proteins are important for the protective humoral immune response since these proteins induce antibodies that neutralize the infectious virus and prevent its spread by cell fusion. However, little is known about cell mediated immune response (CMI). Although a competent CMI response is required to clear the acute viral infection, no data is available concerning the role of individual structural proteins for the induction of protective CMI response in man.
The monotypic nature of the virus is illustrated by epidemiological observations showing that a single infection gives lifelong immunity. Further, it has been experienced that protection against disease can be obtained by the use of same vaccine throughout the world. These characteristics emphasize the stability of major antigenic sites on the surface component, the haemagglutinin (H), and fusion protein (F) protein. However, variations in antigenic characteristics of internal components, the matrix (M), polymerase (P) and nucleoproteins (N) might occur. Hooshmand Sheshbaradaran, and Norrby used monoclonal antibodies against five structural components of measles virus to determine the degree of antigenic variation within these proteins amongst virus strains of measles virus (four fresh wild type isolates, two vaccine and two laboratory strains and a strain derived from a case of subacute sclerosing panencephalitis) giving lytic interactions in cell culture. The major surface proteins showed limited variations in their epitopes between the nine strains. No variations in the fusion (F) protein and only three variations in the haemagglutinin (H) protein epitopes were detected by radioimmune precipitation assay and other serological tests using a panel of eleven monoclonal antibodies against each protein. The two innermost proteins, the nucleocapsid and polymerase protein, also appeared to be antigenically stable as no variation was detected between strains using, in each case, a panel of six hybridomas. In sharp contrast, the epitopes on the matrix (M) proteins of different strains showed extensive variations in their reactivity with the nine anti-M monoclonal antibodies (Sheshbaradaran and Norrby).

5. NUCLEOCAPSID PROTEINS

The nucleocapsid protein (P3) has an approximate molecular weight of 60,000. However, it is susceptible to trypsin treatment and can be degraded into two components of molecular weight 38,000 and 24,000 respectively (Bussel et al, 1974).
6. MEMBRANE PROTEINS

These are non-glycosylated proteins obtained from treatment of the virus with Triton X-100 at lower salt concentration. These proteins are insoluble at low ionic strength (Hall and Martin, 1974b).

7. SURFACE PROTEINS

The chemical structure of viruses can often be studied by the stepwise degradation of the particle by the use of proteolytic enzymes. In case of enveloped viruses, this has been achieved by treatment with proteolytic enzymes such as bromelain or by detergents such as Tween-20, Triton X 100 or NP 140 (Maeno et al, 1970; Chen et al, 1971). Hall and Martin showed that when purified measles virus was incubated with bromelain at 37°C for 4 hours and the mixture centrifuged at 65,000 g for 1 hour at 4°C the pellets obtained were devoid of outer projections. Also, the supernatants obtained in such experiments contained most of the HA activity, whereas HL and cell fusion activities were both destroyed. The polypeptide composition of the spikeless components showed that four main proteins were present, none of which was labeled with tritiated glucosamine. Hence, it would appear that the glycoproteins are involved in the spike structure and are associated with HA activity. The size of bromelain released components were estimated by fractionation on sucrose gradients and by gel filtration on sephadex G-200. Sedimentation in the presence of catalase showed that the virus material was approximately 6 S, while the gel filtration showed that the glycoprotein had a molecular weight of about 1,20,000. These results suggest that the two glycoproteins present in the envelope are associated strongly, even after release from the virus (Hall and Martin, 1974b).
CLINICAL DESCRIPTION OF DISEASE

The sequence of events between exposure to measles virus and subsequent primary acute illness in the normal course has been extensively studied, described and reviewed (Robins F.C., 1962; Kempe CH, 1965; Krugman, 1965; and Cherry JD, 1987). Based on information from both monkeys and humans, first there is localized infection of the respiratory epithelium of the nasopharynx and possibly the conjunctivae, with spread to regional lymphatics. Further events then occur in a manner similar to those observed in Fenner ectromeliamaouse experimental mode (Fenner, 1948). Specifically 2-3 days following exposure, primary viremia develops with further replication of virus at the site of inoculation as well as in the regional and distant reticuloendothelial tissue. Then, 5-7 days following exposure, there is an intense secondary viremia of 4-7 days duration that leads to infection of and further replication in the skin, conjunctivae, respiratory tract and other distant organs. The amount of virus in the blood and infected tissue peaks 11-14 days after exposure and falls off rapidly over the next 2-3 days (Preblud and Katz).

These events correspond with an incubation period, a prodromal stage and finally the rash and complications of measles infection. The incubation period, i.e. the interval between exposure and symptoms, is usually 10-12 days. If infection occurs by the parenteral route, the incubation period is shortened by 2-4 days (Roboins F.C., 1962; Kempe, 1965).

The prodromal stage is heralded by the onset of fever, malaise, conjunctivitis, coryza and tracheobronchitis manifesting as cough and it lasts for 2-4 days. This symptom is not unlike with an upper respiratory tract infection. The temperature rises during the ensuing 4 days and may be as high as 40.6°C (105°F). Koplic spots, the exanthem believed to be pathognomonic of measles, appears on the buccal mucosa 1-2 days before rash onset and may be noted for an additional 1-2 days after rash onset. The rash is an erythematos
maculopapular eruption that usually appears 14 days after exposure and spreads from the head (face, forehead, hairlines, ears and upper neck) to the extremities over a 4-8 days period. The exanthem is usually most confluent on the face and the upper body and initially blanched on pressure. Over the next 3-4 days, the rash fades in the order of appearance and assumes nonblanching brownish appearance. Desquamation can be detected in areas of greatest involvement. Other constitutional signs and symptoms such as anorexia, diarrhoea and generalized lymphadenopathy may be present.

Virus can be isolated from both the nasopharynx and blood during the later part of the incubation period and during early stages of rash development (Gresser and Chany, 1954; Enders and Peebles, 1954). While the virus can be isolated from the urine as late as 4 days after the rash onset, the viremia generally clears 2-3 days after rash onset in parallel with the appearance of antibody. Individuals with measles are considered to be infectious 2-4 days before and 4 days after rash onset.

Recovery from infection is associated with the production of interferon and secretary antibodies as well as the establishment of cellular immunity. Although subclinical infection with boosting of antibody may occur with subsequent exposure, the immunity following natural infection is believed to be lifelong (Bech V., 1959).

The complications associated with measles infection have been subject to much description and review (Cherry J.D., 1987). In industrialized countries the most commonly cited complications associated with measles infection are otitis media (7 to 9 %), pneumonia (1 to 6 %), post-infection encephalitis (1/1,000 to 1/2,000 cases of measles), subacute sclerosing panencephalitis i.e. SSPE (1/1,00,000 cases), and death (1/10,000 cases). Complications are more likely to be present if the fever has not lysed within 1-2 days of rash
onset. The risk of serious complications and death is in young children and adults. Pneumonia, which is responsible for approximately 60% of deaths, is more common in younger patients, whereas acute encephalitis is more frequent in adults (Black F.L., 1982). Some other described complications include thrombocytopenia, laryngitis, hepatitis, appendicitis and ileocoititis, pericarditis and myocarditis, glomerulonephritis, hypercalcemia and Stevens and Johnson syndrome while it has been long assumed that measles infection exacerbates or activates tuberculosis; it is no longer certain that, this is the case (Black F.L., 1982; Barkin R.M., 1975).

Measles infection runs a devastating course in children in developing countries where the mortality rate is estimated to be as high as 10% (Krugman S.L., Katz S.L. 1985; Cherry J.D., 1987). The rash is intense, often haemorrhagic (black measles) and resolves after marked Desquamation. Inflammation of mucosa leads to stomatitis and diarrhoea. The later is a frequent cause of death, since it may persist long after the acute infection and further aggravate a preexisting malnutritional state. The combination of vitamin A deficiency and keratitis results in high incidence of blindness (Morely D., Wood and M., Martin W. J., 1963). Of particular interest is SSPE, which is a rare degenerative central nervous system disease caused by a persistent infection with a defective measles like virus (Choppin P.W., 1981). Patients develop progressive personality changes, myoclonic seizures and motor disability. They lapse into coma and subsequently die. The average age of onset is 9 years. Male patients outnumber female patients 2:1 to 4:1. Although patients with SSPE have high titres of measles specific antibodies in the sera and cerebrospinal fluid, there appears to be a relative lack of synthesis of antibody against one of the six structural polypeptides, the M protein (Choppin, Richardson & Merz, 1981).

Unlike rubella, there is no convincing evidence that maternal infection with measles in the first trimester is associated with
increased risk of miscarriage and prematurity (Siegel M., 1973; Young N.A. & Gershon A.A., 1983). The typical course of measles as described can be modified by the presence of antibody (Krugman & Katz). This situation usually arises in the infants with residual maternal transplacental antibody or in the individual given immunoglobulin. A second clinical measles infection, however, may occur if immunity is incomplete. An atypical variant of measles occurs in some recipients of killed measles vaccine who are at risk for developing severe delayed hypersensitivity reactions following exposure to wild virus (Krugman & Katz; Cherry J.D.; Krugman S.). Patients with a typical measles lack antibody to the measles virus F protein and have exaggerated cellular responses to measles antigen (Choppin, 1981; Choppin & Richardson, 1981). Exposure results in an unbalanced response between cellular and humoral immunity with production of extremely high levels of measles specific antibody. After an incubation period of 1-2 weeks, a prodrome consisting of high fever, headache, abdominal pain, myalgia and cough ensues. In the next 2-3 days an unusual rash erupts on the extremities and spreads centripetally. While the exanthem might be erythematous and maculopapular, it is frequently petechial or vesicular and is accompanied by edema. The illness is frequently mistaken for rocky mountain spotted fever and must also be differentiated from meningococcemia and drug induced eruptions (Neiberg et al, 1980; Brooks et al, 1981).