



Cell Mediated And Humoral Immune Responses To Mumps Virus: Recent Developments

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ABSTRACT

Despite the extensive use of Mumps virus vaccines for almost forty years, there is still a paucity of knowledge concerning the Host-Pathogen Interaction. This paper reviews the advances in our knowledge of the antiviral immune response with particular reference to the selection of attenuated mumps virus strains used in different vaccine formulations. The ability of mumps viruses to evade the interferon component of the immune response is described in detail. This study also presents recent findings from our laboratory and others concerning the humoral and cell mediated responses to mumps virus. Particular attention is placed on mumps virus induction of interleukin-10 (IL-10) and the potential for this to be a novel immune evasion strategy is discussed. Finally, recent advances in our understanding of the immune response to mumps virus are placed in the broader context of our current understanding of this disease.

INTRODUCTION:

Mumps virus, like other members of the family Paramyxoviridae, is an enveloped virus with a non-segmented single stranded RNA genome of negative polarity. Mumps is an acute communicable disease usually characterised by fever and parotid swelling, although other organ systems, particularly the central nervous system (CNS) and glandular tissue, may be involved. Although the immune response to mumps virus has been explored in some detail, it is surprising how little definitive knowledge we possess. For example the precise roles of the antibody and cell mediated immune responses have not been fully elucidated [1, 2]. This review will place recent advances in our knowledge of the antiviral immune response in the broader context of our current understanding of this important childhood pathogen.

HISTORY:

The name “ mumps” may derive from an old English verb that means “ to grimace, grin, or mumble” , and was the name given to an epidemic illness, first described by Hippocrates in the 5th century B.C. [3]. He characterised this disease by swelling near the ears, which was occasionally accompanied by painful swelling of one or both testes. In 1790, Robert Hamilton, an English physician was the first to associate the involvement of the CNS with mumps, describing the neuropathology of a fatal case [4]. However, it wasn’ t until 1935, that Johnson and Goodpasture convincingly demonstrated that mumps was the result of a viral infection. They showed that filtered parotid secretions from a number of patients infected with mumps could induce parotitis in Rhesus monkeys, when injected into the Stenson

ducts [5]. They later went on to fulfil Koch's postulates, by inoculating a number of children with a filtrate of infected monkey parotid tissue, an experiment that would struggle to meet modern regulatory approval. Six out of the thirteen children developed noticeable parotid swelling [6]. In 1945, Habel cultured the virus in chick embryos, thus allowing a more practical alternative for the propagation and study of this infectious disease [7].

NOMENCLATURE, PHYSICAL & CHEMICAL PROPERTIES OF THE VIRUS:

The *Paramyxoviridae* include important pathogens of infants and children, including parainfluenza viruses (PIV), mumps virus (MuV), measles virus (MV), and respiratory syncytial virus (RSV), as well as newly discovered viruses such as Nipah virus [8, 9]. The family *Paramyxoviridae* was reclassified in 2000 into subfamilies the *Paramyxovirinae* and the *Pneumovirinae*. *Paramyxovirinae* contains three genera Respirovirus, Rubulavirus and Morbillivirus (Fig. 1).

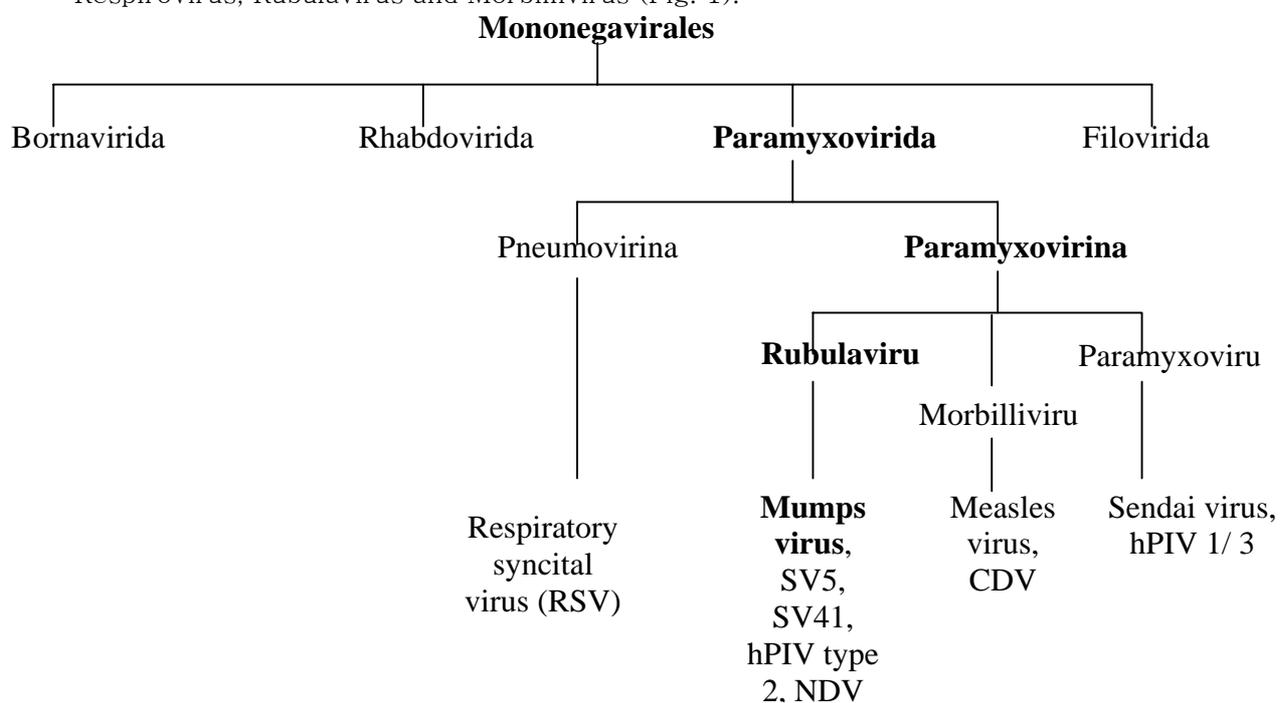


Figure 1. Phylogenetic relationship of the Mononegavirales viruses. Mononegavirales are negative sense, single stranded RNA viruses, which consist of four virus families.

Originally, mumps virus was classified with influenza viruses in the myxovirus family, but has since been reassigned to the genus Rubulavirus, based on nucleocapsid morphology, genome organisation, and biological properties of the proteins. Other members of the Rubulavirus genus include simian virus 5 (SV5), parainfluenza virus (PIV) types 2 and 4, and Newcastle disease virus (NDV) [10].

Paramyxovirus virions are pleomorphic, enveloped particles with surface glycoprotein spikes, projecting from this envelope. Within the envelope a large helical nucleocapsid encases a single-stranded, non-segmented, negative-sense RNA genome. The genome is complementary in its base sequence to the positive-sense viral mRNA that can be located in infected cells [11].

Mumps virions range in size from 100-600nm and contain seven major structural proteins, whose genetic organisation has been determined, to be 3'-NP-P-M-F-SH-HN-L-5' [12](Table 1). The most abundant component is the nucleoprotein (NP), which determines the helical structure of the nucleocapsid. A

phosphoprotein (P) and another high molecular weight (L) protein, are also associated with the nucleocapsid, and may be related to the enzymatic functions of RNA replication [12]. A membrane, or matrix (M) protein forms the structure that underlies the viral envelope, and is involved in virion assembly during the replication cycle. The main viral envelope is composed of two glycoprotein spikes, an attachment protein, hemagglutinin-neuraminidase (HN) and a fusion protein (F) [10]. HN has three functions: it recognises sialic acid-containing receptors on cell surfaces; it promotes the fusion activity of the F protein, thus allowing the virus to penetrate the cell surface; and it acts as a neuraminidase, removing sialic acid from progeny virus particles to prevent self-agglutination [13]. The fusion protein is thought to be directly responsible for the fusion of viral envelope, with the cellular plasma membrane.

The multifunctional character of the HN molecule makes it an attractive target for structure-based design of antiviral drugs. The structure of the HN protein of NDV has been reported [14]. However, these structural studies failed to reveal a second sialic acid binding site. Much of the literature favours two separate sites for the HA and the NA functions. From a drug design standpoint, a single site in HN would provide an attractive target for inhibiting cell attachment, fusion promotion and NA activities, thereby blocking both, infection by the virus and the virus spreading [14].

The small hydrophobic (SH) protein is most likely a membrane protein although its functions unknown [15]. The phylogenetic relationships among mumps virus strains have been determined using SH gene sequences. Interestingly this has revealed the SH protein as the most variable part of the mumps virus genome [16-18], a finding which may have far reaching consequences for future subunit vaccine design.

ANIMAL MODELS:

The only natural host of mumps virus is man. Propagation of mumps virus through cell lines has increased our understanding of virus infectivity. However, while, *in vitro* experiments are a necessary part of our understanding, *in vivo* experiments allow a greater understanding of host pathogen interaction. Experimentally induced mumps infections have been reported in the monkey [5-6], however the adaptation of mumps virus to hosts other than primates has been rarely resulted in symptomatic disease other than when Wollenstein infected cats and induced parotitis, orchitis and meningitis [19-21]. Cabasso attempted to adapt mumps virus to guinea pigs, hamsters, mice, cotton rats, and rabbits utilising several routes, but all produced negative results [22]. Although the latter animals are not susceptible to infection, they may respond immunologically to the injection of live or killed virus. [22]. Infection has been reported in other species notably the dog [23], ferret [24], guinea pig [25], adult and suckling hamster [26, 27], mouse [28], and the developing chick embryo [7]. Of these animal models, the suckling hamster has attracted most attention. The neuroadapted Kilham strain of mumps virus produces lethal encephalitis in newborn hamsters [29-32]. This strain was originally isolated from human breast milk, and produced severe meningoencephalitis in suckling hamsters on intracerebral inoculation [30, 33]. Wolinsky et al. have demonstrated that the neuroadapted Kilham strain caused systemic infection, including the CNS in newborn hamsters by intraperitoneal inoculation [34].

Using mice instead of hamsters is more beneficial, because there exists a greater knowledge of their genetic and immunological backgrounds. Mice have been central to modern understanding of T cell function (Fig. 2). Hosaka et al. reported that intraperitoneal injection of an egg-adapted Urabe AM9 virus vaccine strain of mumps into mice generated a memory cytotoxic T lymphocyte response (CTL) [35].

However, while there is a greater knowledge of the murine immune system, they have proved problematic for the study of this paramyxovirus. In our studies of MuV immunogenicity we find no evidence of virus replication, at least in immunocompetent mouse strains. This absence of viral replication may limit the applicability of the mouse model, however it is also possible that murine studies will uncover subtle viral effects that could be masked by the pathology associated with replicating virus.

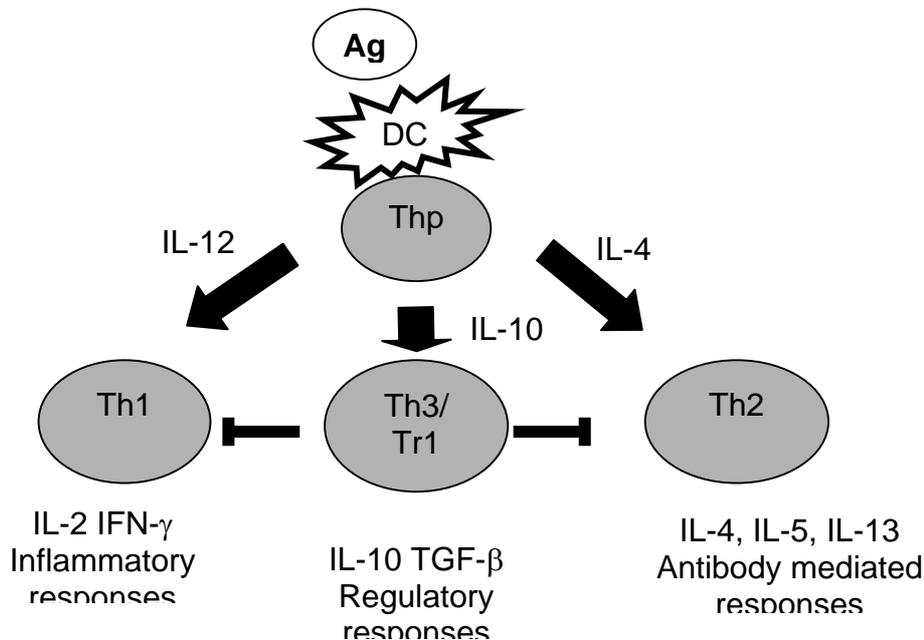


Figure 2. Polarization of helper T cells (Th) and their cross-regulatory cytokines. Antigen (Ag) encounter by dendritic cells (DC) results in T cell priming. The phenotype of the response is conditioned by cytokines such as IL-12 present during early infection. Mumps induction of IL-10 may influence this.

PATHOGENESIS & PATHOLOGY:

Although many animals can be readily infected experimentally, natural infection with mumps virus is restricted to humans. It is transmitted by droplet spread or direct contact [5]. The primary site of replication is located in the mucosal epithelium of the upper respiratory tract, which spreads to the local draining lymph nodes. The incubation period from exposure to first clinical symptoms is about 18 days [36]. Secondary replication occurs in the respiratory tract, followed by generalised viraemia and dissemination to other organs with replication in endothelial cells [37]. Lymphocytic infiltration and destruction of periductal cells lead to blockage of the ducts in both the salivary glands and the seminiferous tubules of the testes, thereby inducing the classic symptoms of mumps virus infection [38]. Other organs may be involved in the course of mumps infection. These include the CNS, liver, pancreas, spleen, kidneys, heart and lungs.

The CNS is a common target for dissemination of mumps virus, and can be estimated from cerebrospinal fluid (CSF) pleocytosis. In at least half of all infections, virus replication can be detected within the CNS [39, 40]. Most CNS involvement includes the meninges and the choroid plexus. Prior to the initiation of mumps vaccination programmes, mumps was the most common cause of childhood deafness and lymphocytic meningitis [38].

Table 1. Physical characteristics of mumps virus components

Viral Component	Size (Amino acids)	Molecular mass (kDa)	Function
NP	549	61-73	Determines helical structure of capsid.
P	391	41-47	Enzymatic functions of RNA replication.
M	375	41-42	Structural & virion assembly.
F	538	65-74	Fusion of viral envelope.
SH	57	-	Unknown.
HN	582	74-80	Viral entry & prevention of self-agglutination.
L	2,261	180-200	Enzymatic functions of RNA replication

CLINICAL FEATURES:

Exposure to mumps virus is followed by an asymptomatic period lasting 14 to 18 days. Virus is present in saliva for several days before the onset of clinical symptoms, and for up to 5 days into the course of the disease [41]. The most characteristic feature of mumps virus infection is that of parotid swelling. This occurs in up to 95% of all symptomatic cases [42]. If swelling is severe, the mouth cannot be opened for pain and tightness, and is dry due to the obstruction to the flow of saliva. This discomfort may persist for up to a week [38]. Orchitis may occur 4-5 days after the onset of parotitis, or may be seen in the absence of parotid swelling.

Lymphocytic or viral meningitis may develop about 5 days following the onset of clinical symptoms. Again CNS involvement can be observed following the onset of parotitis, but also in the absence of parotitis [43]. In the CSF, there is a marked increase in proteins and lymphocytes, and mumps virions can be isolated in the first few days. The onset of mumps meningitis is marked by, fever with vomiting, neck stiffness, headache and lethargy [44]. Fatality due to mumps infection stands between 1.6 and 3.8 per 10000 of mumps cases [45].

TREATMENT, PREVENTION & CONTROL:

No specific antiviral chemotherapy is effective against mumps virus. A patient who presents with uncomplicated parotitis does not need special treatment. Symptomatic treatment includes simple analgesics, but for the severe pain of orchitis, morphia may be required. Corticosteroids are recommended in severe cases of parotitis, more especially in orchitis [38]. During World War II Gellis et al. carried out a controlled study where adult males presenting with parotitis were given either an intramuscular injection of 20ml of γ -globulin prepared from human convalescent serum or simply confined to hospital for routine non-specific symptomatic therapy

[46]. A partial protective effect was seen with γ -globulin obtained from convalescent donor serum [46]. The outcome of the study suggested that antiviral immunotherapy with γ -globulin was of limited utility but could be useful in selected cases early in the course of the disease.

Control measures for mumps virus are primarily directed at prevention, which have been achieved through immunisation strategies. Various attempts to vaccinate against mumps have been made over the last hundred years. In one early study, Johnson and Goodpasture sprayed material containing parotid gland extracts bearing live virus into the nose and oral cavity in an attempt to immunise monkeys [47]. It was only in the 1960s that the first live attenuated mumps vaccine, Jeryl Lynn B, was developed. It originated as an egg allantoic fluid isolate from the daughter of a prominent virologist [48]. And was licensed for use in the USA in 1967. By 1992, this vaccine had been administered to approximately 135 million children and adults around the world. This resulted in the steady decline of reported mumps cases. However, the use of mumps vaccine remains relatively restricted to more affluent countries.

There is a desirable trend in vaccine protocols to combine more components in fewer administrations to children. Part of this process saw the initiation of vaccination programmes against measles, mumps, and rubella (MMR) throughout Europe in the late 1980s. MMR immunisation is now part of national vaccination campaigns for healthy children in most parts of the world. It is usually performed during the second year of life with a second dose at 4-6 years or 11-12 years, depending on national health policies. It has been recommended that infant immunisation with live attenuated measles virus should be carried out at 9 months of age [49]. However, Ceyhan et al. studied the efficacy of a single dose MMR delivered at 12 months of age, compared to monovalent measles vaccination at 9 months followed by MMR revaccination at 15 months of age. Their results indicated a higher rate of vaccine failure (30.1%) after early measles vaccination followed by MMR at 15 months of age, than MMR vaccination at 12 months of age (9.7%). It is known that maternal antibodies may reduce the seroconversion rates to live measles virus in infants under 12 months of age [50]. It is likely that the passive transfer maternal antibodies and the consequent interference with MMR take, is the main reason for primary vaccine failure [51]. Nevertheless it may be possible to devise novel strategies that will allow neonatal immunization [52].

STRAIN SELECTION & VACCINE DESIGN:

As prevention of mumps infection is the only approach to controlling this disease it is worth outlining some of the controversies surrounding vaccine design and the individual attenuated strains employed. There have been five mumps vaccine strains in common use, detailed below.

Jeryl Lynn mumps vaccine strain. The Jeryl Lynn strain was developed in the USA by passaging isolated virus in embryonated hen's eggs, then in chick embryo cell cultures [48]. It has been reported that this isolate is in fact a mixture of two strains JL isolates 2 and 5 [53]. Clinical studies showed that a single dose of Jeryl Lynn vaccine leads to initial seroconversion rates of 80-100% [54]. Further studies show that the Jeryl Lynn vaccine invokes long-lived immune response. In 1982, when mass vaccination began in Sweden, using the Jeryl Lynn vaccine, 73% of 229 children who were immunised with MMR at 18 months of age remained seropositive a decade later [55]. Despite the continuing mass vaccination with this strain, the less neuropathogenic SBL-1 strain of the mumps genotype A remains endemic in Sweden [56, 57]. With regard to stability of the attenuated phenotype, a 10 year retrospective study of hospitalised cases of mumps in the USA, found one case of aseptic meningitis

per 100 000 doses of MMR vaccine in a group of children aged 12-23 months [58]. Overall, vaccines based on the attenuated Jeryl Lynn strain have been shown to afford a higher seroconversion rate, with few adverse events.

Leningrad-3 mumps vaccine strain. This vaccine strain was developed in the former Soviet Union in guinea-pig kidney cell culture, with further passages in Japanese quail embryo cultures. Vaccines using the Leningrad-3 strain have been used since 1974 in the former Soviet Union and other countries. Studies have shown that children aged 1-7 years have a seroconversion rate of 89-98%, and a protective efficacy of 92-99% [59]. Further retrospective review of the medical records of Slovenian patients hospitalised with aseptic meningitis during 1979-1986 found a high incidence of 1 case per 1000 doses of a combined measles, mumps (Leningrad-3) vaccine, however all symptoms are reported to have resolved with no further complications [60]. Nevertheless this incidence prompted the search for improved attenuated strains.

Leningrad-Zagreb mumps vaccine strain. This vaccine was developed in Croatia, by further attenuation of the Leningrad-3 strain by adaptation and passage on chick embryo fibroblast cell culture. Studies in Croatia showed 87-100% seroconversion to L-Zagreb mumps vaccine and efficacy of 97-100% [61]. In 1993, Tesovic et al. reported an equally high incidence of 1 case of aseptic meningitis for each 1000 doses of MMR (L-Zagreb strain), among children vaccinated at a median age of 14 months [62]. In Brazil, da Cunha et al. examined the occurrence of aseptic meningitis and mumps as an adverse event of L-Zagreb-MMR vaccine. They showed a marked increase in the number of notified cases of aseptic meningitis, 3-4 weeks after mass immunisation with L-Zagreb-MMR [63].

Considering that Leningrad-Zagreb vaccine strain was developed by further passage of Leningrad-3 virus vaccine strain, the high incidence of aseptic meningitis may not be surprising. Clearly, a 0.1% chance of CNS involvement associated with a vaccine is not desirable, and is one reason for the limited application of these vaccine formulations.

Rubini mumps vaccine strain. This vaccine was isolated from the urine of a child by isolation in the human diploid cell W138 that was subsequently passaged through embryonated hen's eggs, and adapted to the MRC-5 human diploid cell line [64]. The Rubini vaccine was licensed in Switzerland in 1985. Recent studies in Switzerland, Italy and Portugal provide evidence that mumps vaccine based on the Rubini strain does not appear to offer long-term protection against mumps infection [65]. Several studies have confirmed the low efficacy of the Rubini vaccine virus strain [66, 67]. A study of secondary attack rates among family contacts of confirmed mumps cases found a protective efficacy of just 6% for the Rubini vaccine, compared with 73% for the Urabe vaccine and 62% for the Jeryl Lynn vaccine [66]. The poor efficacy of this strain is borne out by the experience in Portugal. In 1992, this country began to immunise with the Rubini MMR vaccine strain exclusively. However, this was followed by a mumps epidemic in 1995-96, with the highest incidence among children aged 1-4 years. This epidemic appeared to be correlated to the introduction of the Rubini vaccine virus strain [68]. In a study carried out in Germany, Tischer et al. looked at the humoral immune response when children were immunised with three different MMR vaccines. They showed that immunisation with MMR containing the Rubini component induced detectable antibody in only 38% of the vaccinees [69]. Whether low antibody titres can protect against disease is unknown, but the fact that only 38% of vaccinees presented detectable antibody, either the initial responses were poor, or that this Rubini strain does not induce lasting memory responses.

Urabe mumps vaccine strain. Urabe AM9 vaccine was produced from a 1967 Japanese isolate, by six passages in chick amniotic cavity followed by two plaque-to-plaque purifications in monolayers of quail embryo fibroblasts [70]. Urabe AM9 mumps

vaccine virus, was combined with measles and rubella vaccine virus into the MMR formulation, and was introduced into Canada and the UK. In Canada, one study found that 5-6 years after one dose of MMR the seropositivity rate was 93%, compared with 85% for the Jeryl Lynn vaccine strain [71]. In the UK, a similar study showed that 4 years after a single dose of MMR vaccine, that seropositivity rates were 85% compared with 81% for the Jeryl Lynn vaccine strain. However, following reports from physicians of aseptic meningitis post vaccination, Canada initiated a series of studies. These showed that the Urabe vaccine strain was also a mixture of viruses [72]. In 1990, Canadian authorities withdrew the license to distribute the MMR vaccine containing the Urabe AM9 mumps vaccine strain, because the casual association of vaccination with the occurrence of aseptic meningitis was established unequivocally [73, 74]. Parallel studies in the United Kingdom have examined aseptic meningitis rates following immunisation with the Urabe virus vaccine strain. One multi-centre study showed a rate of 9 aseptic meningitis cases per 100 000 vaccine doses [75]. These and other studies led to suspension of vaccine distribution in the UK in 1992. Likewise, Japan undertook a nationwide surveillance in 1989, demonstrating an overall rate of 49 cases of aseptic meningitis per 100 000 doses of vaccine [76]. The distribution of the domestically produced MMR vaccine containing the Urabe mumps strain was suspended from use in 1994.

Despite these drawbacks it must be appreciated that well over 100 million doses of mumps-virus containing vaccines have been delivered in the U.S., with few adverse reactions [77]. Those events that are reported are rash, pruritus and purpura, which tend to be allergic in nature and frequently, resolve themselves. A survey of the literature by the Vaccine Safety Committee of the Institute of Medicine failed to find any relationship between the administration of mumps virus containing vaccines and the development of encephalopathy, encephalitis, Guillain-Barre Syndrome, or Type I insulin-dependent diabetes mellitus [78]. Considering the scale of the mass vaccination programme this is a powerful indication of the safety and beneficial nature of the mumps component of these vaccines.

Recently, it has been postulated that MMR vaccination might be linked with a reported increase in autism in some countries or with atypical bowel diseases [79]. This has prompted a great deal of scientific examination and also public anxiety in some countries. The results of this extensive examination failed to find any evidence to support either claim [80-84]. The result of the initial claims has been a decline in vaccine coverage in some countries with the result that many children are now at risk of measles, mumps and rubella, and that the possibility of eradication of measles has been delayed.

HOST-PATHOGEN INTERACTION:

Improved vaccine formulations will only come from an enhanced understanding of both the host and the virus. Given the short generation times of paramyxoviruses, a rapid host response is essential and must be aimed at several different levels. The portal of entry for mumps virus is the airway mucosa so clearly the virus has developed strategies to overcome the mechanical protection afforded by muco-ciliary clearance. Natural killer (NK) cells are known to be significant effector components of the innate immune system, which aid in the initial defence against viral infection both via direct cellular cytotoxicity and by the production of inflammatory cytokines. In mice, NK cell-mediated cytotoxicity and IFN- γ production occur early in the course of infection by RSV, and the presence of NK cells is associated with the subsequent influx of CD8⁺ T cells [85]. Nevertheless, NK cells seem to afford only limited early defence against mumps virus, Clearly an important early immune system

task is to containing infection prior to the development of adaptive immunity and prevent overwhelming infection. This function is usually ascribed to interferon (IFN).

Interferons are a family of cytokines that were discovered as soluble factors that mediate cellular resistance to virus infection. They can be classified into two groups; type I and II. Type I IFNs, may be produced by any cell in response to viral infection, whereas type II IFN, is produced in response to the recognition of infected cells by activated T and NK cells. The antiviral functions of IFN are mediated by induction of IFN-stimulated genes (ISGs) such as 2', 5'-oligoadenylate synthase (2-5AS), and dsRNA activated protein kinase (PKR) [86]. Fujii et al. reported that various cell lines, which were persistently infected with mumps virus, exhibited a poor induction of 2-5AS and PKR [87, 88]. However, not only have paramyxoviruses been shown to disrupt the interferon signalling pathway, Sendai virus (SeV) infection also renders both human and murine cells unresponsive to IFN- α / β [89]. STAT proteins are activated by the JAKs after stimulation with appropriate cytokines. Mice deficient in STAT1 and STAT2 proteins correlate with inactivation of the IFN pathway [90, 91]. A decrease in STAT-1 α , rather than the down-regulation of IFN-receptors, IRF-1 and IRF-2, causes the suppression of these IFN-induced genes [92].

The anti-IFN strategies employed by viruses to circumvent the interferon response include, (1) inhibition of functions of IFN-induced antiviral proteins, (2) suppression of interferon production and (3) interference with IFN signal transduction [93-95]. It is not surprising given the pathogenic nature of mumps virus that it too has evolved strategies to counter the effect of IFNs.

Most paramyxoviruses encode the P, V, and C proteins from the same gene by using alternative initiation codons and mRNA editing mechanisms [96]. The P protein is a structural component of the viral RNA polymerase, whereas V and C proteins are virus-encoded accessory factors whose function in some paramyxoviruses is inhibition of the IFN α / β signalling pathway. Studies on the V protein of SV5 (simian virus 5) by Didcock et al. showed that the STAT1 level decline from around 4 hours after infection and eventually become undetectable [97]. The ability of the SV5 V protein to degrade STAT1 and block IFN signalling was confirmed by Andrejeva et al., using 2fTGH cells constitutively expressing SV5 V protein. These results demonstrated that the SV5 V protein blocks IFN signalling by targeting STAT1 for proteasome mediated degradation [98]. The cysteine-rich V protein of mumps virus inhibits the JAK/STAT pathway by inducing the degradation of STAT1 [92, 99, 100]. (Fig. 3).

It is interesting to note that STAT1 is targeted by most of the rubulaviruses. STAT1 is not only a critical component of the IFN signalling pathway, but also serves as a molecule involved in the constitutive expression of certain genes including caspases [101, 102], and low molecular polypeptide 2 (LMP2) [103]. LMP2 is a subunit of the proteasome involved in the processing of T-cell antigens. MHC class-I antigen is necessary for the presentation of endogenous virus antigens on cell surface membranes. It is well known that the IFN-g signalling pathway through induction or activation of the ISGF-3 complex regulates MHC class-I expression. Thus, inactivation of STAT-1 α could be correlated with a decrease in MHC class-I expression. This decrease allows cells persistently infected with mumps virus to escape host immunosurveillance [99].

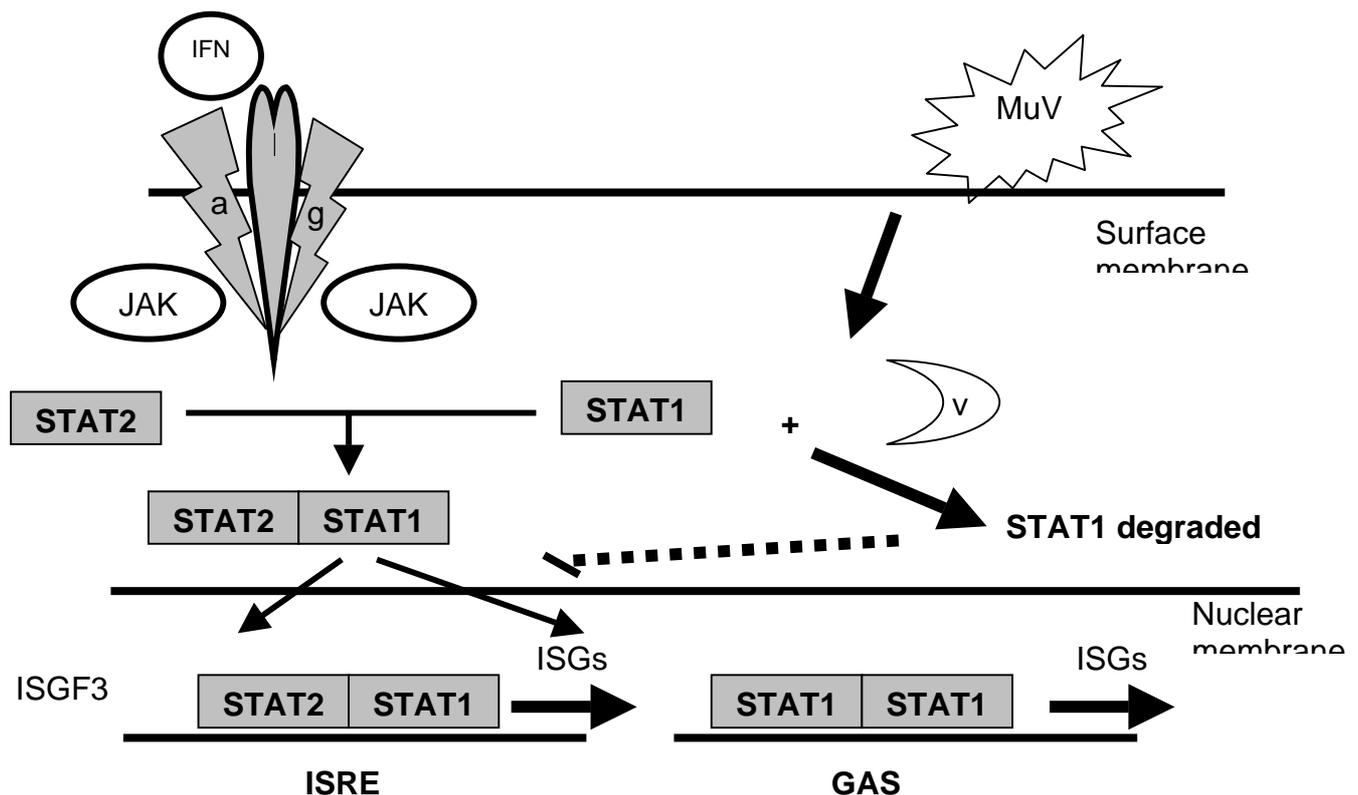


Figure 3. Disruption of Interferon (IFN) signalling pathways. Paramyxoviruses inhibit signalling by degrading STAT-1. The cysteine rich V protein of Mumps is a candidate for this function. ISRE=Interferon secreting response element, GAS = Interferon gamma activated site, ISG=Interferon secretion genes.

ANTIBODY RESPONSES TO MUMPS VIRUS:

Protection against virus infection is determined by cell-mediated and humoral immune responses. Humoral immunity is mediated by antibody. The simplest and most direct way in which antibodies can protect from pathogens is by binding and blocking access to permissive target cells (neutralisation). However antibody may work in a wide variety of ways that includes opsonization, activation of complement, countering viremia, and through mediating antibody-dependent cell-mediated cytotoxicity.

The role of the specific humoral response, to mumps virus, as a defence factor has not been definitively explained [1]. Several authors have shown that mumps virus elicits the production of specific antibodies IgM and IgG [104, 105]. High serum levels of the specific antibodies IgM and IgG are evidence of the exposure of the immune system to viral antigens. During the course of immunisation with MMR, the primary immune response to mumps is marked by the increase in specific IgM antibodies. While administration of a second dose of MMR vaccine elicits a large boost in IgG antibody levels, with little or no IgM response: the so-called anamnestic or secondary response [106].

It might be expected that the early invasion of the salivary glands and other organs, should induce sufficient specific antibody to prevent the spread of the virus, and counter potential meningitis, the most frequent serious complication in the natural course of infection. However, Johnstone et al. provided evidence to the contrary [107]. Comparing patients with mumps meningitis with or without parotid

involvement, high titres of antibodies directed against internal ribonucleoprotein core soluble antigen than high titres of antibodies directed against envelope antigen [107].

Attempts to dissect the role of antibody have been hampered by the difficulties surrounding the animal models described above. For example in our early studies, murine immunisation by intra muscular and intra peritoneal, using alum as an adjuvant, or by the intra nasal route with doses as high as 1/10 human dose failed to induce serum or mucosal antibody responses. Even when purified mumps virus was delivered in complete Freund's adjuvant by the intra muscular route little or no specific IgG could be detected, although CD4⁺ T cell responses were induced (see below).

The measurement of immune responses to mumps virus remains a controversial issue. Determination of mumps virus specific antibodies in human sera is hampered by false positive results, due to the fact that, there is considerable cross-reactivity between mumps virus and other paramyxoviruses [108]. Nevertheless, a variety of assays for antibodies can be applied successfully to sera to demonstrate whether an individual has either been exposed to infection or responded to vaccination. However, quantitative assays are required, if the levels of immunity are to be estimated, and for mumps these remain problematic [109]. Pipkin et al. described assays based on plaque reduction, microtitre plate neutralization, haemagglutination inhibition, and ELISA. They reported that the antibody titres measured by HI or ELISA was poorly predictive of each other or of the neutralising antibody titer. ELISA titers were markedly higher than in neutralising titer as might be expected, and some individuals seroconverted by ELISA but not by neutralisation [109]. Christenson and Bottiger reported similar results [110]. The serum antibody titer in response to vaccination is typically lower than that observed in children naturally infected with mumps [111]. If antibody is the protective mechanism then there is a possibility that vaccinees may become infected at a later date. However, the difficulty in obtaining accurate results from humans, who are the natural hosts of mumps virus, doesn't bode well for researchers investigating mumps virus immunogenicity in non-permissive hosts.

It has been reported that secretory antibody is produced in saliva after natural mumps infection, and that most of the neutralising antibody is of the IgA class [112, 113]. Since local secretory neutralising antibodies act directly, Tanaka et al. have gone on to show that nasal antibody is important in protecting the host against mumps infection [114]. They showed that secretory IgA (sIgA) with neutralising activity was produced in the nasal cavity after vaccination as well as after natural mumps infection. Presence of nasopharyngeal antibody suggests that the memory of the local immune system in the nasal cavity, which is the initial site for the invasion and replication of mumps virus, was well maintained. Cusi et al. discovered that intranasal immunisation with mumps virus DNA vaccine delivered by influenza virosomes into mice, elicits mucosal and systemic immunity [115]. IgA antibodies were detected in faeces of mice 7-10 days after the last immunisation, which indicated that intranasal immunisation of DNA induces mucosal immunity at the enteric site [115].

Studies with murine monoclonal antibodies suggest that the hemagglutinin-neuraminidase protein (HN) is the major target for the humoral response to mumps virus infection [116]. Cusi et al. localised a neutralising epitope on the mumps virus hemagglutinin-neuraminidase protein [117]. Their aim was to characterise further putative neutralising epitopes by using sera of mice previously immunised with fragments of HN protein and to confirm and localise those putative epitopes on the HN protein by using synthetic peptides [117]. They discovered that a fragment of the HN protein spanning aa 329-335 could, not only be relevant for the virulence of mumps virus but also for the induction of neutralising antibodies. This fragment was

shown to have the ability to induce neutralising antibodies not only to the attenuated virus strain, but also a wild-type strain. The presence of antibodies to the HN of mumps virus in the mucosa is important, since they represent barrier immunity [115]. Thus the HN protein represents the major target to induce a protective humoral response to mumps virus [118].

CELL-MEDIATED RESPONSES TO MUMPS VIRUS:

Ziola et al. demonstrated that T-cell cross-reactivity occurs among members of the three genera comprising the *Paramyxoviridae*. He demonstrated that the *Paramyxoviridae* cross-react at the T-cell level, but not at the antibody level [119]. T-cell reactivity between mumps virus and parainfluenza virus, is expected as these viruses are serologically related [120]. However, Ziola et al. uncovered a strong bi-directional T-cell cross-reactivity between respiratory syncytial virus (RSV), measles virus and mumps virus [119]. RSV typically infects children before one year of age [121], and thus many children will have acquired T-cell immunity before they reach the recommended age for MMR vaccination (12-15 mths). This means that RSV-induced T-cells, through cross reactivity with mumps and measles virus antigens, could influence subsequent development of immunity to either or both of these vaccine viruses [119].

On entering the target cell, mumps virus is not accessible to antibodies. Therefore cell mediated immunity mediated by CD4⁺ T helper (T_H) or CD8⁺ cytotoxic T cells (CTL) are essential in the elimination of viruses [122]. Cell mediated reactions depend on direct interactions between specific T cells and target or antigen presenting cells which display the specific antigen in the context of either class I or II MHC molecules. However, it is known that rubulaviruses target STAT1 for degradation (Fig 3.), which may be correlated to a decrease in MHC class-I expression [99]. The decrease results in reduced CTL activation, thus favouring mumps virus replication and dissemination.

The role of cellular immunity in infection with mumps virus is supported by data derived from experimental animals [2], as well as from humans. Mumps meningitis is a common complication of mumps virus infections, and MuV-specific CTL accumulated in the CNS can be detected in cerebrospinal fluid (CSF) [123]. Nagai et al. demonstrated that at the acute phase of mumps meningitis, CD8⁺ and HLA-DR⁺ cells increased in the CSF, and that cytotoxic-suppressor T cells activated by MuV antigens may accumulate [124]. Kimura et al. were the first to focus on the T-cell receptor (TCR) gene repertoire in patients with infectious CNS diseases. They evaluated TCR Va gene expression in T lymphocytes from patients with mumps meningitis, and showed that the use in the CSF was widespread but biased toward three or less families in each patient. The biased use of the Va gene suggests that T cells with dominant Va genes may be mumps-specific T lymphocytes, which were selectively recruited to the CNS [125].

A major goal of vaccine development has been to design viral vectors that elicit CTL that can efficiently clear an ongoing or subsequent infection. Parks et al. showed that recombinant Simian virus-5 (rSV5) expressing chicken ovalbumin, was capable of eliciting a robust CTL response [126]. More importantly, vaccination with rSV5-Ova was found to efficiently elicit high avidity CTL. This is an extremely desirable attribute of a vaccine vector, as high avidity CTL have been shown to be optimal for virus clearance [127].

Investigations by Cusi et al. demonstrated that immunisation with a mumps virus DNA vaccine delivered by virosomes elicited not only mucosal immunity but systemic cell-mediated immunity too [115]. They carried out proliferation assays, which predominantly measure CD4⁺ T-cell responses. Cells from mice immunised

with live mumps induced mainly IL-10 and, to a lesser extent, IL-2. IFN- γ was not seen in any of their samples, which may be explained by the fact that mumps virus holds the ability to suppress the IFN system [100]. When Cusi et al. immunised via the mucosal route with mumps virus HN-virosomes a Th2 response was observed, while immunisation with mumps virus F-virosomes characterised a Th1 response. A combination of these two vaccines containing both mumps virus HN and F proteins could be necessary for a vaccine capable of inducing cellular and humoral immunity [115].

Our own studies of murine immunisation with MMR could only elicit responses when powerful adjuvants were employed. However, mumps virus specific polyclonal T cells lines could be derived from these mice (Fig. 4A). Analysis of these lines demonstrated MuV-specific proliferation and cytokine release. Interestingly IL-4, IL-5 and IFN- γ were not detectable. However significant levels of IL-10 were elicited in response to mumps virus stimulation. Thus even following a powerful immunisation regimen, mumps virus is only sparingly immunogenic, and seems to favour IL-10 induction. Cusi et al. also discovered that when purified live mumps virus was inoculated into BALB/c mice IL-10 was the principal cytokine induced [115]. To probe these responses further and to understand the mechanism of viral modulation within the murine model, it will be necessary to derive mono-specific T cell clones.

In preliminary studies, we have derived a panel of MuV-specific CD4⁺ T cell clones. Results of two representative clones are shown (Fig. 4A). Clone MuV1.1 proliferates and secretes high levels of IFN- γ , with little or no IL-4, IL-5 or IL-10 produced. High levels of IFN- γ drive the characteristic T_H1 response needed to destroy intracellular parasites. Secretion of IFN- γ by MuV1.1 does not appear to be consistent with previous studies [115]. However, individual proteins of mumps virus may have the ability to elicit opposing T cell responses. Cusi et al. demonstrated that the F protein when entrapped in influenza virosomes secreted increased amounts of IgG2a and IL-2, suggestive of a Th1 response, yet they observed no IFN- γ secretion [115]. Taken together these results suggest that at least under some conditions, mumps virus immunisation can induce IFN- γ . In contrast to MuV1.1, a second clone, MuV3.1 proliferated and secreted IL-5 and high levels of IL-10, but little or no IFN- γ in response to mumps virus (Fig4B). This is consistent with the response observed by Cusi et al. They showed that when HN was entrapped in influenza it stimulated a T_H2 response, production of IgG1 and IL-10 [115]. Further characterisation of both MuV-specific T cell clones by FACS analysis confirmed that they are CD3⁺CD4⁺CD8⁻ T cells (Fig 5).

Interestingly, both Cusi et al and our studies showed high levels of IL-10. This may be a feature of the mouse, which is not permissive to MuV replication. However, it may be that different MuV antigens provoke different cytokine profiles [115]. Cusi et al. suggested that IL-10 was indicative of a T_H2 response. However, recent work on IL-10 suggests that it is modulatory, acting as a regulatory or suppressor function during an immune response. It is known that Paramyxoviruses overcome the antiviral properties of IFNs, while other viruses have evolved immunosuppressive strategies by encoding homologues of immunomodulatory cytokines, including IL-10. It may be that induction of host IL-10 by mumps virus is an additional mechanism to evade host immunity. A number of intriguing possibilities arise from the data described above. It is clear that attenuated mumps virus delivered alone or as MMR can generate T_H1 and T_H2 specific profiles. The observation of high levels of IL-10 seen by Cusi and also presented here suggests a viral evasion strategy in which mumps induces IL-10 in order to suppress protective cell mediated responses [115]. However, an alternative hypothesis may be worthy of investigation. Mumps viremia is associated with fever presumably due to endogenous cytokines such as IL-1. The induction of IL-10 by

mumps may in fact be a protective mechanism, by dampening the immune response. The virus may counter the potentially pathological influence of inflammation. Mumps like other viruses requires time to replicate and disseminate from the host to others. It is counter productive for the virus to allow overwhelming inflammation, which would compromise the host and its own spread. IL-10 may be beneficial to both virus and the survival of the host. While the latter possibility is startling it will require animal models and particularly gene knockout models to determine which of these roles IL-10 promotes. The viral components that generate the suppressive environment will also need to be identified and removed from future subunit vaccines as they may suppress active immune responses to viral antigens of mumps virus and perhaps other components.

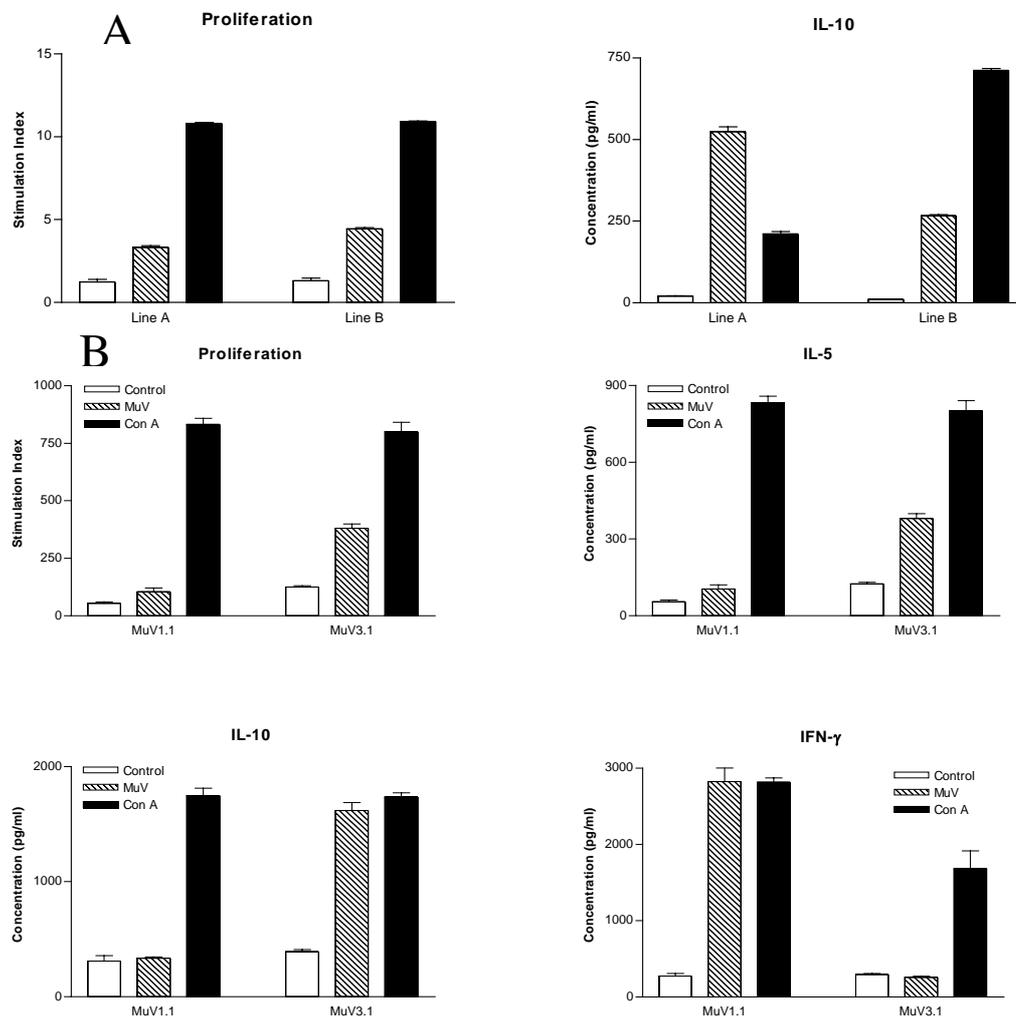


Figure 4. Murine CD4⁺ Th response to MuV immunization. A) Two Mumps virus specific T cell lines were established after a single round of in vitro restimulation. Proliferation and IL-10 secretion are shown in response to control (open bar), MuV (hatched bar) or Mitogen positive control (Black bar). B) Characterization of Two T cell clones MuV1.1 and MuV 3.1 in response to control, virus or mitogen stimulation, with regard to Proliferation, or IL-5, IL-10 or IFN-g secretion

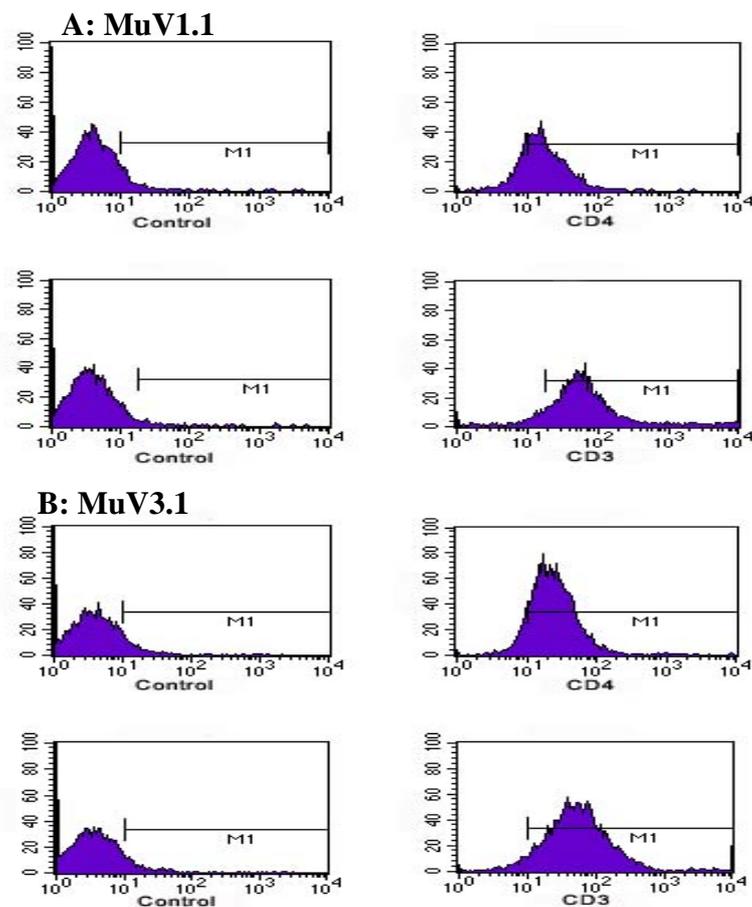


Figure 5. Characterization of MuV specific clones by FACS analysis as CD4+ T cells. A Clone MuV1.1 is a Th1 type clone whereas (B) Clone MuV3.1 has a Tr 1 phenotype.

Conclusions:

Safe and effective combination vaccines, containing live attenuated mumps virus strains, have been in use around the world since the early 1980s. However, while mumps has been documented since the 5th century B.C. there is still little understanding of the complexity of the immune response it generates. The mouse has opened the gateway to our understanding of the human immune system and how it responds to invading pathogens. Examination of mumps virus is hitherto been hampered by the fact that viral replication is restricted to humans and select primates[6, 128]. However, the mouse and mumps-specific T cell clones may prove more beneficial in uncovering viral mechanisms of immune evasion. The time is right for a renewed interest in the fundamental biology of mumps virus and how it interacts with permissive and non-permissive hosts. Such fundamental knowledge will be essential if we are to design new vaccines by rational criteria.

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