Class I-restricted CTL induction by mucosal immunization with naked DNA encoding measles virus haemagglutinin

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We have investigated the class I-restricted CTL response specific for measles virus haemagglutinin (HA) in the spleens of mice immunized by various mucosal routes with a DNA plasmid carrying the HA gene (pV,j-HA). A single immunization with recombinant DNA injected in the buccal mucosa induced an HA-specific CTL response. Similarly, nasal immunization with the DNA vaccine induced primary CTLs against measles virus HA. Booster immunization did not enhance the CTL activity. Oral or intrajejunal immunization with the plasmid induced a CTL response of lower magnitude. However, this could be potentiated by co-administration of the mucosal adjuvant cholera toxin or cationic lipids (DOTAP). These data show that a CTL response can be generated by mucosal vaccination using DNA vaccines.

Measles remains a major public health problem, causing the deaths of at least 1 million children annually in endemic regions. The lack of success of the present attenuated vaccine in developing countries is in part due to acquired maternal antibodies, which persist in infants until the age of 9 to 12 months (Albrecht et al., 1977). Furthermore, the current live vaccine cannot be safely administered to immunocompromised children, and does not provide protection at mucosal surfaces which are known to be the portal of entry and primary site of replication of this virus (Walker, 1994).

DNA vaccination using recombinant plasmids encoding viral proteins is a promising alternative to the use of live vaccines. These vectors induce both humoral and cell-mediated immune responses to viral antigens in a way that mimics natural infection (Schödel et al., 1994). Nucleic acid immunization was first reported to require targeting of the vector to long-lived cells such as myoblasts (Pertmer et al., 1995; Ulmer et al., 1993). However, more recent studies suggest that DNA vectors can provoke an immune response when delivered by a mucosal route, inducing humoral immunity (Fynan et al., 1993; Hyde et al., 1993; Ban et al., 1996). Furthermore, targeting of the vector to professional antigen-presenting cells (APC), such as skin Langerhans cells, can be achieved by gene-gun inoculation, which enhances the immune response (Fynan et al., 1993; Pertmer et al., 1995).

We examined the capacity of a plasmid (pV,j-HA) encoding the haemagglutinin (HA) gene of measles virus to induce an HA-specific CTL response when administered by various mucosal routes. The plasmid was constructed by subcloning a cDNA encoding MV-HA (Gerald et al., 1986) into the BglII site of plasmid pV,j (Montgomery et al., 1993), expression from which is driven by the CMV promoter. Site-directed mutagenesis was used to introduce BamHI sites at the 5’ and the 3’ end of the MV-HA cDNA and to optimize the Kozak sequences of the ATGs (Kozak, 1986) in order to enhance expression of the encoded HA. The palindromic immunostimulatory sequences (Sato et al., 1996) are present in pV,j-HA.

We have previously reported that intramuscular immunization with the same vector induces HA-specific, Th1-biased humoral immunity and a class I-restricted specific CTL response (Cardoso et al., 1996). In the present study, mucosal immunizations were performed with a single dose of 100 µg of pV,j-HA, in BALB/c mice. This was given either by the nasal route (under light ether anaesthesia), the oral route (by gastric intubation) or the enteric route (by intrajejunal injection, under laparotomy). Since the buccal mucosa is covered by a network of dendritic cells analogous to skin Langerhans cells (Ahlfors & Czerkinsky, 1991), we also performed buccal immunizations, consisting of transepithelial injection of 100 µg of pV,j-HA in the buccal mucosa.

Cholera toxin (CT) is a powerful mucosal adjuvant (Holmgren et al., 1993). Its adjuvant effect on the HA-specific CTL response to pV,j-HA was thus investigated by co-administration of the toxin with the vector. We also tested whether the anti-HA CTL response could be enhanced by addition of the cationic lipid DOTAP (Boehringer Mannheim) to pV,j-HA prior to vaccination.

Mice were sacrificed 10 days after immunization, and the HA-specific CTL activity in their spleens was analysed as previously described (Beauverger et al., 1993). Briefly, spleen

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Fig. 1. Class I-restricted HA-specific CTL response induced in the spleens of BALB/c mice immunized once (b, c, d, e) or twice (a) with 100 µg of pVJ-HA either alone ([square]), with 5 µg CT ([circle]) or with 25 µg DOTAP ([triangle]). Ten days after the last immunization, mice were sacrificed and spleen cells were restimulated for 7 days in bulk cultures with P815–HA transfectants. Cytotoxic activity was measured by 51Cr release assay, using P815–HA and P815 cells as targets. The results are expressed as the percentage of HA-specific lysis, by subtracting the percentage (background) lysis of P815 target cells from the percentage lysis of P815–HA transfectant cells. The data are from one representative experiment out of three, using four mice per group. Background lysis never exceeded 20% of the positive control.

As a positive control, we gave mice two intramuscular injections of 100 µg of pVJ-HA. This induced high levels of CTL activity in the spleen, reaching 60% of HA-specific lysis at an effector to target (E:T) ratio of 30:1 (Fig. 1a). In parallel, we examined the CTL response induced by pVJ-HA administered by various mucosal routes. A single nasal immunization with 100 µg of pVJ-HA induced a primary HA-specific CTL response in the spleen. The CTL activity reached 30% of specific lysis at an E:T ratio of 30:1 (Fig. 1b), and was not increased by further immunizations (Fig. 2). Co-administration of CT (5 µg) did not increase the CTL response. This result is in contrast with previous observations demonstrating an adjuvant effect of CT on the systemic CTL response after nasal immunization with a vaccinia–HA recombinant virus (Etchart et al., 1996) and suggests that presentation of antigens expressed by DNA plasmids and viral recombinants may involve distinct pathways. Addition of DOTAP to the plasmid did not increase the CTL response. Although cationic lipids have been shown to enhance DNA uptake and transgene expression by epithelial cells of the lower respiratory tract (Hyde et al., 1993), they did not increase influenza virus-specific antibody responses after nasal immunization with recombinant DNA (Ban et al., 1996).

A single 100 µg dose of pVJ-HA administered by the intragastric route induced a weaker splenic CTL response than obtained by the nasal route (Fig. 1c). However, contrary to observations made after nasal immunizations, co-administration of CT with pVJ-HA intra-gastrically significantly enhanced the anti-HA CTL response (P < 0.01, as calculated by Student’s t-test, at an E:T ratio of 10:1). This increase was also observed when DOTAP was mixed with the plasmid prior to administration (P < 0.05). Similar results were obtained after direct injection of pVJ-HA into the gut lumen (intrajejunal immunization) (Fig. 1d). This response was also increased with CT (P < 0.05), and even more so with DOTAP (P < 0.001). The observation that similar levels of measles virus-specific CTLs are generated after either oral (intragastric) or intra-jejunal
CTLs induced by mucosal DNA vaccination

**Fig. 2.** HA-specific cytotoxicity in the spleens of mice immunized once (a), or twice within 21 days (b), by the nasal route with 100 µg of pVj-HA either alone (■), with 5 µg CT (□) or with 25 µg DOTAP (△). Results are expressed as described in the legend to Fig. 1.

Injection of naked DNA strongly supports the hypothesis that the DNA vector is not degraded in the stomach. Thus, DNA-based vectors may be efficient in oral immunization, compared with live virus vectors which are often highly sensitive to gastric degradation (Etchart et al., 1996; Meitin et al., 1994).

A single immunization with pVj-HA injected into the buccal mucosa also induced an anti-HA CTL response (Fig. 1e). This pertains to the presence within the buccal epithelium of functional antigen-presenting dendritic cells (Eriksson et al., 1996). It should be noted that it is not possible to compare the efficiency of buccal immunization with 100 µg of plasmid injected into the buccal mucosa with the same dose delivered to the gut lumen or via the gastric route, where significant amounts of plasmid can be washed out. The observation that CT, co-injected with the plasmid via the buccal route, did not enhance the CTL response in the spleen may be attributed to its selective adjuvant effect on mucosa-associated lymphoid tissues (MALT) but not on pluristratified epithelia, such as that of the buccal mucosa.

Little is known concerning the APC type which captures and expresses DNA vaccines. DNA delivered through the skin (Hengge et al., 1996) or by the nasal route (Hyde et al., 1993) is transiently expressed by tissue-specific epithelial cells. However, studies on the intramuscular injection of recombinant DNA in bone-marrow chimeric mice demonstrated DNA expression by myoblasts but antigen presentation by donor type haematopoietic cells (Cort et al., 1996; Ulmer et al., 1996a, b). Several lines of evidence support the hypothesis that dendritic cells (DC) are involved in the presentation of proteins encoded by DNA vectors delivered by the mucosal route. DC capture antigens from epithelial tissues and migrate to draining lymph nodes for antigen presentation to T cells, in vivo. They also appear to act in vitro as potent APC for primary CTL induction after DNA transfection (Rouse et al., 1994). Furthermore, the buccal mucosa contains a network of DC analogous to that of the skin Langerhans cells, and is an inductive site for T cell-mediated immune responses (Ahlfors & Czerkinsky, 1991). We have previously reported that buccal DC are endowed with APC function and the capacity to activate HA-specific CD8+ T cells following local immunization with a viral peptide (Eriksson et al., 1996). In addition, mucosae of the respiratory and gastro-intestinal tract also contain DC, which have been reported to act as efficient APC for mucosally delivered protein antigens (Liu & MacPherson, 1993; Holt et al., 1994).

Taken together, these data show that mucosal immunization using a single dose of naked DNA is efficient for the induction of specific CTLs against measles virus, and presents a promising new approach for mass immunization against this virus.

**References**


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