Antiviral Research, 19 (1992) 275–284

AVR 00586

A vaccine against Semliki Forest virus consisting of a monoclonal anti-idiotypic antibody cross-linked to a protein which contains virus-specific T-helper cell epitopes

C.A. Kraaijeveld, T.A.M. Oosterlaken, A. Snijders, B.J. Benaissa-Trouw, G.L. Ekstijn and H. Snippe

Eijkman-Winkler Laboratory for Medical Microbiology, University of Utrecht, Utrecht, The Netherlands

(Received 23 March 1992; accepted 27 May 1992)

Summary

A recombinantly expressed protein, consisting of cro-β-galactosidase at the N-terminus and amino acid residues 115 to 151 of the E2 membrane of Semliki Forest virus (SFV) at the C-terminus containing two T-helper cell epitopes of SFV, was cross-linked with glutaraldehyde to a noninternal image monoclonal anti-idiotypic antibody (ab2α MAb) able to induce SFV-neutralizing anti-anti-idiotypic (ab3) antibodies in BALB/c mice. This vaccine, which might potentially induce SFV-specific T-helper cell memory, established in BALB/c mice a state of protective immunity against virulent SFV within 10 days of immunization. A steady rise in serum neutralization titre occurred from day 7 to day 28 after primary anti-idiotypic immunization, levelling off thereafter. In primarily immunized mice significant rises of serum neutralization titres, which could be indicative for an operational T-helper cell memory, were not observed after challenge on day 35 with virulent SFV. The results suggest that SFV is neutralized by ab3 antibodies shortly after challenge, preventing, thereby, virus multiplication to levels sufficient to provoke a measurable booster response.

Semliki Forest virus; Vaccine; Monoclonal anti-idiotypic antibody; Anti-anti-idiotypic antibody; T-helper epitope; Quil A

Correspondence to: C.A. Kraaijeveld, Eijkman-Winkler Laboratory for Medical Microbiology, Heidelberglaan 100, G04.614, 3584 CX Utrecht, The Netherlands.

Introduction

Recently we identified a noninternal image monoclonal anti-idiotypic antibody (ab2α MAb), designated 1.13A321 (IgG1), that, cross-linked by glutaraldehyde to keyhole limpet haemocyanin (KLH) and combined with the adjuvant Quil A, evokes Semliki Forest virus (SFV) neutralizing anti-anti-idiotypic (ab3) antibodies upon immunization of BALB/c mice (Oosterlaken et al., 1991a; Oosterlaken et al., 1992). Cross-linking syngeneic immunoglobulins to a foreign protein, such as KLH, promotes a stronger anti-idiotypic immune response, presumably due to provision of T-helper cell epitopes of KLH (Rimmelzwaan et al., 1989). From the viewpoint of vaccine development, however, KLH is a nonrelevant carrier molecule, provoking unnecessary immune reactivities and, therefore, we designed an alternative approach in which SFV-specific T-helper cell epitopes were provided. Instead of KLH a recombinantly expressed protein consisting of cro-β-galactosidase and amino acid residues 115 to 151 of the E2 membrane glycoprotein of SFV at the C-terminus (Snijders et al., 1989) was prepared and used as carrier. The SFV fragment in the recombinant protein contains two T-helper cell epitopes (Snijders et al., 1992). Immunization of mice with recombinant protein alone evokes delayed type hypersensitivity (DTH) against whole SFV (Snijders et al., 1989) but neither neutralizing antibodies nor protective immunity (unpublished results). The SFV-specific DTH-inducing T-helper epitope is thought to be immunodominant and located at positions 137–151 (Snijders et al., 1992). The usefulness of recombinant protein as carrier molecule to ab2 MAb 1.13A321 is investigated in a mouse model.

Materials and Methods

Virus

The avirulent prototype strain of SFV (Garoff et al., 1980) was obtained from H. Garoff, The Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden. It was used for determination of SFV neutralizing antibodies. The virulent strain of SFV, SF/LS 10 C1/A (Bradish, Allner and Maber, 1971), was received from C.J. Bradish, The Porton Down Microbiological Research Establishment, Salisbury, UK. The 50% lethal dose (LD_{50}) for 10–14 week-old BALB/c mice proved to be 1 to 2 plaque-forming units (PFU) when intraperitoneally (i.p.) injected in 0.5 ml phosphate-buffered saline (PBS) at pH 7.2.

Cells and media

L cells, a continuous line of mouse fibroblasts, were maintained in Dulbecco's modified Eagle's medium (DMEM), buffered with 0.01 M *N*-2-hydroxyethylpiperazine-*N'*-ethane sulfonic acid, supplemented with 5% calf serum, 0.2% tryptose and antibiotics.

SFV-specific MAb

SFV-neutralizing MAb UM 5.1 (IgG2a), conjugated to horseradish peroxidase (HRPO) by the periodate method (Nakane and Kawaoi, 1974), was used for detection of SFV in cell culture (Van Tiel et al., 1986).

Anti-idiotypic MAb

Anti-idiotypic (ab2) MAb 1.13A321 (IgG1) was induced against SFV-neutralizing MAb UM 1.13 as described previously (Oosterlaken et al., 1991a). Ab2 MAb 21.1A1 (IgG2a), specific for encephalomyocarditis virus (EMCV) neutralizing MAb UM 21.1 was used as a control (unpublished results; Vlaspolder et al., 1988).

Carrier molecules for ab2 MAb 1.13A321

The conventional carrier keyhole limpet haemocyanin (KLH) was purchased from Calbiochem, La Jolla, CA, USA.

Cro-β-galactosidase and a recombinantly expressed protein, consisting of cro-β-galactosidase at the N-terminus and amino acid residues 115 to 151 of the E2 membrane protein of SFV at the C-terminus were prepared and purified as previously described (Snijders et al., 1989). The SFV fragment contains two T-helper cell epitopes located at positions 115–129 and 137–151 (Snijders et al., 1992).

Coupling of ab2 MAb to KLH, cro-β-galactosidase and recombinant protein

Ab2 MAb 1.13A321 was purified by protein G Sepharose affinity chromatography (Äkerström et al., 1985) and subsequently coupled to either KLH, cro-β-galactosidase or recombinantly expressed protein. Antibody was covalently conjugated to either carrier molecule with glutaraldehyde, as described previously (Oosterlaken et al., 1988). In brief, 0.8 mg purified 1.13A321 in 0.2 ml PBS was mixed with 1 mg of either substance (in 0.2 ml distilled water) and then covalently coupled to each other by the addition of 0.06 ml of 2.5% glutaraldehyde. After 20 min incubation at room temperature the reaction was stopped with 0.06 ml of 0.2 M glycine. After addition of 0.48 ml distilled water, the mixture was dialysed overnight at 4°C against distilled water to remove excess glycine. Thereafter, the conjugate (1.0 ml) was used for anti-idiotypic immunization of mice.

Mice

Female BALB/c mice were obtained from the National Institute of Health and Environmental Protection (RIVM), Bilthoven, The Netherlands. The mice were kept in the animal house of the University of Utrecht until use at an age of 10 to 14 weeks.

Anti-anti-idiotypic immunization

Mice received a single intracutaneous immunization of conjugated ab2 MAb 1.13A321 (equivalent to 40 μg ab2 MAb per animal) combined with the

adjuvant Quil A (50 μl per animal). Quil A, a purified saponin (Morein et al., 1984; Kensil et al., 1991), was obtained from Superfos Biosector, Denmark. The mixture (0.1 ml) was injected at two sites (2 × 0.05 ml) in the vicinity of the draining lymph nodes in the groins. Blood for determination of SFV-neutralizing antibodies in serum was obtained from ether-anaesthetized mice by retro-orbital puncture.

Determination of SFV-neutralizing antibodies

SFV-neutralizing antibodies were determined by neutralization enzyme immunoassay (N-EIA), as described earlier with slight modifications (Van Tiel et al., 1986). In brief, serum samples obtained from individual mice were serially diluted in DMEM, supplemented with 5% calf serum, in wells of 96-well plates. Each serum dilution (0.05 ml) was mixed with a standard infectious dose of 1000 PFU of SFV (0.05 ml) and incubated for 1 h at 37°C. Subsequently 20 000 L cells (0.1 ml) were seeded into each well to form monolayers. Non-neutralized SFV was allowed to multiply for 18 h at 37°C. Then the L cell monolayers were fixed by addition of 0.05% glutaraldehyde for 10 min at room temperature. After washing with tapwater and rinsing with PBS direct EIA of SFV was performed with HRPO-labelled MAb UM 5.1. Preincubation of the virus inoculum with SFV-neutralizing serum reduces virus multiplication and, thereby, the appearance of the spike proteins on the surface of L cells, as indicated by inhibition of absorbance in the EIA. Inhibition of virus multiplication by immune serum could be calculated as a percentage of control: % inhibition ('virus neutralization') = 100% − [(A_{450} serum − A_{450} cell control)/(A_{450} virus control − A_{450} cell control)] × 100%. The titer of immune serum can be arbitrarily defined as the dilution causing 50% inhibition. Absorbance values were measured at 450 nm with a Titertek multiscan photometer (Flow Laboratories, Irvine, UK).

Protection of mice

Immunized mice and control mice were intraperitoneally injected with 400 PFU (equivalent to 250 LD_{50} units) of the virulent strain of SFV in 0.5 ml PBS (pH 7.2). To quantitate protection mice were observed for 21 days; nonprotected mice died within eight days after challenge.

Results

Anti-idiotypic immunization of BALB/c mice with ab2 MAb 1.13A321 evokes SFV-neutralizing ab3 antibodies in serum that could be blocked by addition of 1.13A321 in contrast to the polyclonal SFV-neutralizing antibodies in normal immune serum (Fig. 1). Even low levels of these SFV-neutralizing ab3 antibodies protected mice against an otherwise lethal SFV infection, as shown in the next experiment that was designed for a dual purpose: to establish how quickly protective immunity evolves after primary anti-idiotypic

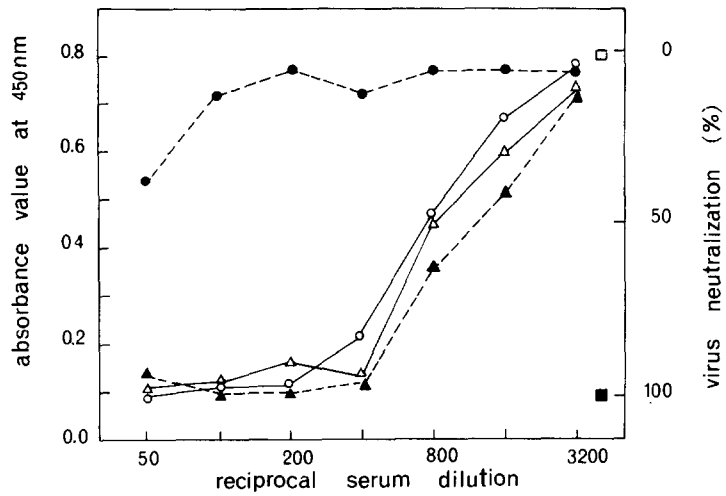

Fig. 1. Absorption by ab2 MAb 1.13A21 of neutralizing activity from anti-idiotype-induced immune serum but not from normal polyclonal immune serum. Dilutions of polyclonal immune serum of an SFV-infected mouse (△, unabsorbed: ▲, absorbed) and immune serum from an anti-idiotype (1.13A321) immunized mouse (○, unabsorbed; ●, absorbed) were mixed with 10 μg of purified ab2 MAb 1.13A321 and incubated for 1 h at 37°C. Thereafter, the neutralizing capacities of unabsorbed and absorbed immune sera were compared by N-EIA. The single symbols at the right of the figure represent the mean ($n=8$) absorbance values of the virus control (□) and the cell control (■). All other absorbance values are means of two determinations.

immunization and to compare the efficacy of different carrier molecules.

Groups ($n=20$) of female BALB/c mice were immunized intracutaneously with ab2 MAb 1.13A321 coupled to either KLH, cro-β-galactosidase or recombinantly expressed protein. Just before infection on either day 3, 5, 7 or 10 blood samples were taken from ether-anaesthetized mice for determination of SFV-neutralizing ab3 antibodies in individual sera. In this particular experiment, using KLH as carrier, full protective immunity was established on day 7 (Table 1). Compared to KLH, recombinant protein was slightly less effective as a carrier molecule and full protective immunity was obtained at day 10. Partial protection was obtained with cro-β-galactosidase as carrier. None of the mock-immunized mice were protected, indicating that protection was due to virus-specific immunity. As indicated in Table 1 even undetectable SFV-neutralizing ab3 antibodies (no neutralizing activity in serum dilution 1/4) at the day of challenge might contribute to protection against virulent SFV. In the next two experiments it was investigated whether the SFV-specific T-helper cell epitopes present in the recombinant protein can contribute to the neutralizing antibody response after infection of primarily immunized mice.

Groups ($n=5$) of female BALB/c mice were intracutaneously immunized with either free ab2 MAb 1.13A321 or the same MAb conjugated to recombinant protein. On day 7, 14, 21, 28 and 35 after primary immunization

TABLE 1

Rapid development of protective immunity to SFV after immunization with noninternal image ab2 MAb 1.13A321. Comparison of the efficacy of carrier molecules KLH, cro-β-galactosidase and recombinantly expressed protein

Anti-anti-idiotypic immunization[a]		Development of neutralizing antibodies (N)[b] and protective immunity (P)[c] to SFV							
Ab2 MAb	Carrier molecule	Day 3		Day 5		Day 7		Day 10	
		N	P	N	P	N	P	N	P
21.1A1	KLH	–	–	–	–	–	–	0/5	0/5
1.13A321	KLH	0/5	0/5	0/5	3/5	2/5	5/5	4/5	5/5
21.1A1	cro-β-galactosidase	–	–	–	–	–	–	0/5	0/5
1.13A321	cro-β-galactosidase	0/5	0/5	0/5	0/5	0/5	3/5	0/5	3/5
21.1A1	recombinant protein	–	–	–	–	–	–	0/5	0/5
1.13A321	recombinant protein	0/5	0/5	0/5	0/5	0/5	3/5	1/5	5/5

[a]Female BALB/c mice were intracutaneously immunized with ab2 MAb, coupled to either KLH, cro-β-galactosidase or recombinant protein combined with Quil A.
[b]Blood was obtained just before infection for determination of SFV-neutralizing antibodies in serum by N-EIA. Given is the ratio of mice with detectable neutralizing antibodies (titers $\geqslant 4$) in serum to the number of mice tested.
[c]Mice were intraperitoneally challenged (after blood sampling) with 400 PFU of virulent SFV. Given is the survival ratio (P) at day 21 of infection.

separate groups of mice immunized with conjugated ab2 MAb were intraperitoneally challenged with 250 LD_{50} units of virulent SFV. Mice who received free ab2 MAb were challenged on day 14 and 28. Just before infection blood was obtained from ether-anesthetized mice to determine SFV-neutralizing ab3 antibodies in serum. As shown in Table 2 mice immunized with the conjugate of 1.13A321 and recombinant protein developed SFV-

TABLE 2

Protective immunity against virulent SFV due to immunization with recombinantly expressed protein cross-linked ab2 MAb 1.13A321[a]

Immunization of mice with conjugated or free Ab2 MAb	Interval between immunization infection (days)	Log_{10} neutralization titre (mean ± S.D.) just before infection	Survival Ratio of mice	Log_{10} neutralization titre (mean ± S.D.) after infection at day		
				5	7	20
Conjugate	7	nd	4/5	–[b]	–	1.6 ± 0.1
Conjugate	14	0.9 ± 0.5	5/5	–	–	1.6 ± 0.5
Free	14	nd	0/5			
Conjugate	21	1.4 ± 0.4	5/5	–	–	1.4 ± 0.2
Conjugate	28	1.8 ± 0.2	5/5	–	–	2.2 ± 0.3
Free	28	nd	0/5	–	–	
Conjugate	35	1.4 ± 0.4	5/5	–	–	1.7 ± 0.2
Conjugate	35	1.9 ± 0.5	12/12	1.8 ± 0.4	2.2 ± 0.4	–

[a]Results of two separate experiments in which immunized BALB/c were i.p. challenged with 250 LD_{50} of virulent SFV.
[b]–, not tested. nd, not detected.

neutralizing antibodies and concomitantly a state of protective immunity. At day 7 of immunization four out of five mice were already protected against an otherwise lethal challenge. From day 7 to day 28 the antibody titer rose steadily, levelling off thereafter. Free ab2 MAb did not evoke either protection or neutralizing antibodies in the short time after immunization, indicating that cross-linking of this ab2 MAb is essential for its efficacy as vaccine. From surviving mice blood was taken 20 days after infection. As shown in Table 2 a booster effect after injection of challenge virus was not obvious. In a second experiment a booster response after SFV challenge on day 35 could not be detected 7 days later (Table 2).

Discussion

In this study it is shown that noninternal image monoclonal anti-idiotypic antibody 1.13A321, cross-linked to KLH, evokes detectable SFV-neutralizing ab3 antibodies in serum of BALB/c mice within 10 days after primary immunization (Table 1). Presumably these SFV-neutralizing ab3 antibodies are similar in neutralizing capacity to ab1 MAb UM 1.13, to which ab2α MAb UM 1.13A321 was prepared. Purified MAb UM 1.13 (5 mg protein per ml) neutralizes SFV strains to titers over 10^6 and a dose of 0.1 μg is sufficient to protect all tested mice against an otherwise lethal infection (unpublished results). So it is possible that a relatively low amount of ab3 antibodies is protective. Furthermore, the adjuvant Quil A and the intracutaneous route of immunization may be advantageous for induction of virus-neutralizing ab3 antibodies (Oosterlaken et al., 1991b). The SFV-neutralizing ab3 antibodies in serum could be blocked in their neutralizing activity by addition of ab2 MAb 1.13A321 (Fig. 1). In contrast, neutralization of SFV by immune serum obtained from SFV-infected mice could not be blocked by ab2 MAb 1.13A321. The lack of blocking is explained by the finding that conventional immune serum to SFV contains antibodies to various neutralization epitopes (Boere et al., 1984; Snijders et al., 1991). The lack of blocking is further illustrated by the finding that the idiotypes of the strongly SFV-neutralizing MAb's UM 1.13 and UM 5.1 are not cross-reactive and, therefore, anti-idiotypic antibodies could only block the homologous MAb (our unpublished results).

The presence of SFV-neutralizing ab3 antibodies in immunized BALB/c mice fully explains the occurrence of protective immunity against virulent SFV as could be argued from previous results: passively transferred conventional immune serum protected recipient mice even when the level of neutralizing antibodies was too low to be detected in the sera of those mice (Kraaijeveld et al., 1986). Furthermore, even non-neutralizing antibodies can protect against SFV, as was found also for Sindbis virus (Boere et al., 1983, 1984, 1985; Schmaljohn et al., 1982).

Free ab2 MAb 1.13A321 does not induce SFV-neutralizing ab3 antibodies. Cross-linking either to itself (our unpublished results) or to carrier molecules

seems to be essential for induction of SFV-neutralizing antibodies by 1.13A321. A noninternal image anti-idiotypic (ab2α) MAb such as 1.13A321 is thought to bind with its antigen-combining site (paratope) to a recurring idiotope located on antigen receptors of B-cells, triggering them to proliferate and maturate to antibody-producing plasma cells (Hiernaux, 1988; Jerne, Roland and Cazenave, 1982; Köhler et al., 1989; Rimmelzwaan et al., 1989; Roitt et al., 1985). Presumably the binding of aggregated ab2α MAbs to individual B-cells provides a stronger signal than binding of free ab2 MAb, which might provoke a negative feedback mechanism on B-cell activation by a second binding of their intact Fc parts with Fcγ receptors on the same B-cells (Wofsy and Goldstein, 1990). Cross-linking of ab2 MAb with glutaraldehyde might inhibit such interaction with Fcγ receptors.

In the present study we demonstrate that, as an alternative to KLH, recombinantly expressed protein, consisting of cro-β-galactosidase and amino acid residues 115–151 of the SFV E2 protein, could be used as a carrier molecule for ab2 MAb 1.13A321. The SFV-derived part of the recombinant protein contains two T-helper cell epitopes, located at positions 115–129 and 137–151 (Snijders et al., 1992). Potentially these T-helper cell epitopes might provide SFV-specific T-cell memory to immunized mice. However, a booster response of SFV-neutralizing antibodies following infection in primary immunized mice could not be demonstrated, which might be due to the direct elimination of the challenge virus by the SFV-neutralizing ab3 antibodies. After infection the virus inoculum must multiply to an immunogenic level required to stimulate polyclonal antibody formation against SFV. In agreement with this hypothesis we showed, in earlier experiments, that passively transferred polyclonal immune serum protects mice against the lethal outcome of SFV infection and that formation of neutralizing antibody upon infection is inversely related to the amount of transferred immune serum (Kraaijeveld et al., 1986). The absence or presence of polyclonal SFV-neutralizing antibodies in serum of infected anti-idiotype immunized mice could be monitored by absorption of post-infection sera with ab2 MAb 1.13A321. Indeed, most mice that possess SFV-neutralizing ab3 antibodies at the moment of challenge do not develop detectable polyclonal SFV-neutralizing antibodies (results not shown).

Although the results do not yield a clear-cut positive answer with regard to an operational SFV-specific T-cell memory, the described approach might be useful to enhance the long-term effectiveness of anti-idiotypic vaccines by induction of virus-specific T-helper cell memory.

References

Åkerström, B., Broding, T., Reis, K. and Björck, L. (1985) Protein G: a powerful tool for binding and detection of monoclonal and polyclonal antibodies. J. Immunol. 135, 2589–2592.

Boere, W.A.M., Benaissa-Trouw, B.J., Harmsen, M., Kraaijeveld, C.A. and Snippe, H. (1983) Neutralizing and non-neutralizing monoclonal antibodies to the E2 glycoprotein of Semliki Forest virus can protect mice from lethal encephalitis. J. Gen. Virol. 64, 1405–1408.

Boere, W.A.M., Benaissa-Trouw, B.J., Harmsen, T., Erich, T., Kraaijeveld, C.A. and Snippe, H. (1985) Mechanisms of monoclonal antibody-mediated protection against virulent Semliki Forest virus. J. Virol. 54, 546–551.

Boere, W.A.M., Harmsen, M., Vinjé, J., Benaissa-Trouw, B.J., Kraaijeveld, C.A. and Snippe, H. (1984) Identification of distinct antigenic determinants on Semliki Forest virus by using monoclonal antibodies with different antiviral activities. J. Virol. 52, 575–582.

Bradish, C.J., Allner, K. and Maber H.B. (1971) The virulence of original and derived strains of Semliki Forest Virus for mice, guinea-pigs and rabbits. J. Gen. Virol. 12, 141–160.

Garoff, H., Frischauf, A.M., Simons, K., Lehrach, H. and Delius, H. (1980) Nucleotide sequence of cDNA coding for Semliki Forest Virus membrane glycoproteins. Nature 288, 236–241.

Hiernaux, J.R. (1988) Idiotypic vaccines and infectious diseases. Inf. and Imm. 56, 1407–1413.

Jerne, N.K., Roland, J. and Cazenave, P.A. (1982) Recurrent idiotopes and internal images. EMBO J. 243–247.

Kensil, C.R., Patel, U.P., Lennick, M. and Marciani, D. (1991) Separation and characterization of saponins with adjuvant activity from *Quillaja saponaria* Molina Cortex. J. Immunol. 146, 431–437.

Köhler, H., Kaveri, S., Kieber-Emmons, T., Morrow, W.J.W., Müller, S. and Raychaudhuri, S. (1989) Idiotypic networks and nature of molecular mimicry: an overview. Methods Enzymol. 178, 3–35.

Kraaijeveld, C.A., Benaissa-Trouw, B.J., Harmsen, M. and Snippe, H. (1986) Adoptive transfer of immunity against virulent Semliki Forest virus with immune spleen cells from mice infected with a virulent Semliki Forest virus. Arch. Virol. 91, 83–92.

Morein, B., Sundquist, B., Höglund, S. Dalsgaard, K. and Osterhaus, A. (1984) ISCOM: a novel structure for antigenic presentation of membrane proteins from enveloped viruses. Nature 308, 457–460.

Nakane, P.K. and Kawaoi, A. (1974) Peroxidase-labelled antibody: a new method of conjugation. J. Histochem. Cytochem. 22, 1084–1091.

Oosterlaken, T.A.M., Harmsen, M., Tangerman, C., Schielen, P., Kraaijeveld, C.A. and Snippe, H. (1988) A neutralization-inhibition enzyme immunoassay for anti-idiotypic antibodies that block monoclonal antibodies neutralizing Semliki Forest Virus. J. Immunol. Methods 115, 255–261.

Oosterlaken, T.A.M., Harmsen, M., Jhagjhoor-Singh, S.S., Kraaijeveld, C.A. and Snippe, H. (1991a) A protective monoclonal anti-idiotypic vaccine to lethal Semliki Forest virus infection in BALB/c mice. J. Virology 65, 98–102.

Oosterlaken, T.A.M., Brandenburg, A., Schielen, P., Fransen, R., Kraaijeveld, C.A. and Snippe, H. (1991b) Efficient induction of Semliki Forest virus and mumps virus neutralizing anti-anti-idiotypic antibodies by use of the adjuvant Quil A. J. Immunol. Methods 136, 169–175.

Oosterlaken, T.A.M., Harmsen, M., Ekstijn, G.L., Kraaijeveld, C.A. and Snippe H. (1992) IgVh-determined genetic restriction of a non-internal image monoclonal anti-idiotypic vaccine against Semliki Forest virus. Immunology 75, 224–231.

Rimmelzwaan, G.F., Bunschoten, E.J., UytdeHaag, F.G.C.M. and Osterhaus, A.D.M.E. (1989) Monoclonal anti-idiotypic antibody vaccines against poliovirus, canine parvovirus, and rabies virus. Methods Enzymol. 178, 375–390.

Roitt, I.M., Thanavala, Y.M., Male, D.K. and Hay, F.C. (1985) Anti-idiotypes as surrogate antigens: structural consideration. Immunol. Today 6, 265–267.

Schmaljohn, A.L., Johnson, E.D., Dalrymple, J.M. and Cole, G.A. (1982) Non-neutralizing monoclonal antibodies can prevent lethal alphavirus encephalitis. Nature (Lond.) 297, 70–72.

Snijders, A., Benaissa-Trouw, B.J., Oosting, J.D., Snippe, H. and Kraaijeveld, C.A. (1989) Identification of a DTH-inducing T-cell epitope on the E2 membrane protein of Semliki Forest Virus. Cellular Immunology 123, 23–35.

Snijders, A., Benaissa-Trouw, B.J., Oosterlaken, T.A.M., Puijk, W.C., Posthumus, W.P.A., Meloen, R.H., Boere, W.A.M., Oosting, J.D., Kraaijeveld, C.A. and Snippe, H. (1991) Identification of linear epitopes on Semliki Forest Virus E2 membrane protein and their effectiveness as a synthetic peptide vaccine. J. Gen. Virology 72, 557–565.

Snijders, A., Benaissa-Trouw, B.J., Visser-Vernooij, H.G., Fernández, I., Snippe, H. and

Kraaijeveld, C.A. (1992) A DTH-inducing T-cell epitope of Semliki Forest virus mediates effective T-helper activity for antibody production. Immunology, in press.

Van Tiel, F.H., Boere, W.A.M., Harmsen, M., Benaissa-Trouw, B.J., Kraaijeveld, C.A. and Snippe, H. (1986) Rapid determination of neutralizing antibodies to Semliki Forest Virus in serum by enzyme immunoassay in cell culture using virus specific monoclonal antibodies. J. Clin. Microbiol. 24, 665–668.

Vlaspolder, F., Kraaijeveld, C.A., Van Buuren, R., Harmsen, M., Benaissa-Trouw, B.J. and Snippe, H. (1988) Prophylaxis and therapy of virulent encephalomyocarditis virus infection in mice by monoclonal antibodies. Arch. Virol. 98, 123–130.

Wofsy, C. and Goldstein, B. (1990) Cross-linking of Fcγ receptors and surface antibodies. J. Immunol. 145, 1814–1825.