Mammalian cell expression of malaria merozoite surface proteins and experimental DNA and RNA immunisation

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Abstract

The gene for a 45 kDa merozoite surface protein (MSA-2) of the human malaria parasite *Plasmodium falciparum* was PCR amplified and cloned into eukaryotic expression vectors VR1012 and pcDNA3 to yield plasmids P1 and P2, respectively. The coding sequences for two N-terminal fragments of the 185 kDa merozoite surface protein (MSA-1) gene were similarly PCR amplified and cloned into vectors VR1020 and VR1012 to yield plasmids P3 and P4, respectively. The MSA-1 signal peptide sequence, present in P4, was replaced with the human tissue plasminogen activator signal sequence in P3. The four plasmids expressed the cloned genes under the control of the cytomegalovirus promoter and carried 3′ bovine growth hormone termination/poly A signals. P1, P3 and P4 also contained the cytomegalovirus intron A enhancer sequence. MSA-1 expression was more readily detected than MSA-2 in Cos cells transfected with P3/P4 and P1/P2 respectively. The MSA-2 gene was also cloned into the phagemid pBluescript IISK$^+$ with and without a 3′ poly A tail composed of 35 A residues. MSA-2 was synthesised in HeLa cells infected with a recombinant vaccinia virus carrying T7 RNA polymerase when MSA-2 recombinant pBluescript was transfected into the cells. Inoculation with P1 intramuscularly or intradermally and with P2 intradermally into rabbits led to the production of antibodies to MSA-2 detectable by immunofluorescence and Western blotting. Antibodies were also produced against MSA-1 after intramuscular/intradermal inoculation with P3 and P4. Inoculation of rabbits with MSA-2 mRNA yielded better antibody titres when a poly A tail was present. Antibody levels were maintained for >9 weeks after the final immunisation. However the immune sera failed to inhibit in vitro parasite growth.

Keywords: DNA vaccine; Eukaryotic gene expression; Malaria; Surface protein; RNA vaccine; (*Plasmodium falciparum*)