Immunisation with Genetically Engineered immunogens from merozoite surface antigens of Plasmodium falciparum and the effects of antibodies on Red cell invadion

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Abstract:

Merozoite surface antigens MSA-1 and MSA-2 have been identified as potential candidate molecules for developing and erythrocyticstage malaria vaccine or the erythrocytic stage component of a multi-stage, multi-immune response vaccine against falciparum malaria. Nucleic acid vaccination is a promising new method for immunizing against infectious diseases. The ability of a Plasmodium falciparum merozoite surface protein synthesized in rabbit cells to elicit malaria-protective antibodies was investigated. The gene for MSA-2 (alternatively termed the glycosylated and myristillated smaller surface antigen, GYMSSA) was cloned into two eukaryotic expression vectors VR1012 and pcDNA3 to yield recombinant plasmids VR1012/GYMSSA and pcDNA3/G. These plasmids expressed the gene under the control of a cytomegalovirus promoter and carried bovine growth hormone termination/poly A signals 3' to the cloned genes. VR1012/GYMSSA contained an additional cytomegalovirus intronic enhancer. Transfection of recombinant plasmids into COS cells resulted in the synthesis of barely detectable quanitities of MSA-2. 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. The synthesis of MSA-2 was readily detected in Hela cells infected with a recombinant vaccinia virus carrying T7 RNA polymerase when MSA-2 recombinant pBluescript was transfected into the cells. Inoculation of VR1012/GYMSSA and pcDNA3/G intramuscularly and intrademally into rabbits led to the production of antibodies against MSA-2. Inoculation of rabbits with MSA-2 mRNA yielded better antibody titres whena a poly A tail was present. Antibodies were detected by immunofluorescence and western blotting titres in the range 10-2 to 10-3. Antibody levels were maintained for>11 weeks after the final immunization. However the antibodies did not demonstrably inhibit erythrocyte invasion by merozoites. These findings illustrate some of the shortcomings as well as the potential of developing nucleic acid vaccines against asexual blood stages. A MSA-1 B cell epitope was expressed in a plant virus, viz. cowpea mosaic virus (CPMV), with the view of investigating its use as an epitope-based vaccine. Peptide P109, corresponding to residues 20-38 in the N-terminal block 1 of the MSA-1 sequence in the K1 isolate, which is highly conserved between different parasite isolates, ws presented between alanine 22 and

proline 23 of the ßB-ßC loop of the small coat protein of CPMV. The recombnant virus was successfully propagated in the black-eyed bean, Vigna unguiculata and was shown to be genetically stable by sequencing reverse transcription polymerase chain reaction (RT-PCR) products from the purified virus. Analysis of purified recombiant virus by SDS-PAGE revealed that 95 percent of small coat protein was apparently cleaved C terminal to the penultimate N terminal P109 amino acid in addition to the cleavage C – terminal 24 amino acids of the S protein. The purified recombinant virus was used to immunize rabbits. Although rabbits antibodies against peptide P109 were also elicited, the antibodies in the sera were largely directed against immunodominant CPMV viral epitopes. The failure of antibodies in the immune sera to bind to natibe MSA-1 in an immunofluoresence assay indicates that the MSA-1. The possible additional cleavage reaction occurring between the last two residues of the MSA-1 P109 epitope may contribute to the inability of the rabbit antibodies to recognize the native epitope on malaria parasites. These findings illustrate a drawback to engineering peptide B cell epitopes in recombinant virus coat proteins. In a different approach, glutathione S-transferase (GST)MSA-2 fusion protein was used to raise antibodies in rabbits and the antibodies were tested for their ability to inhibit merozoite invasion of erythrocytes. The MSA-2 gene, isolated by PCR amplification, was subcloned into the bacterial expression vector Pgex-3X to obtain a fusion protein of MSA-2 with Schistosoma japonicum GST. The recombinant protein was used to immunize rabbits, to yield sera that had western blotting and immunofluorescence antibody titres of 10-6. The effects of immune sera, and 1gG, F(ab)'2, F(ab)'2,F(ab) antibodies prepared from the immune sera, on reinvasion and growth of P. falciparum in vitro was examined by microscopy and a [3H]-hypoxanthine incorporation assay. The antibodies did not significantly inhibit erythrocyte invasion or parasite growth when added to cultures as 10 percent v/v serum or as immunoglobulin preparations at concentrations up to 200 µas determined by the Students't test. However in the presence of IgG or F(ab)'2 antibodies, but not preimmune IgG or F(ab) antibodies to MSA-2, the proportion of erythrocytes invaded by more than one merozoite increased significantly as determined by chi square analysis. Multiple invasion is attributed to merozoites, cross-linked by bivalent antibodies, attaching to and subsequently invading the same erythrocyte. The results also demonstrate that merozoites have evolved mechanisms to avoid complement mediated damage. This findings casts doubt on the usefulness of full length MSA-2 as a candidate molecule for inclusion in a malaria vaccine. Despite the failure of anti MSA-2 as a antibodies to inhibit merozoite invasion and parasite growth in vitro, it is possible that the antibodies act in concert with macrophage/monocytes or neutrophils to damage malaria parasites in vivo.

KEY WORDS : Malaria-immunology / Malaria-genetics / Plasmodium falciparum