Cellular and humoral immune responses to a recombinant *P. falciparum* CS protein in sporozoite-immunized rodents and human volunteers

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The immune responses of sporozoite-immunized rodents and of human volunteers exposed to multiple bites of irradiated Plasmodium falciparum *infected mosquitos have been investigated using a yeast-derived recombinant* P. falciparum *circumsporozoite (rPfCS) protein.*

The murine immune response to immunization with rPfCS was not genetically restricted. Nine different murine haplotypes, when immunized with rPfCS, developed high levels of antisporozoite antibodies detectable by IFA and RIA. In addition, injection of rPfCS induced a secondary antibody response in P. falciparum *sporozoite-primed mice. Murine T-cell epitopes were mapped in the C terminus of the rPfCS protein using overlapping synthetic peptides.*

The human T-cell response was investigated using T-cell clones derived from peripheral blood lymphocytes (PBL) of a P. falciparum *sporozoite-immunized volunteer. A total of 40 CD4$^+$ T-cell clones were obtained. Stimulation indices ranged from 2.5 to 103.4 following challenge with rPfCS in the presence, but not in the absence, of antigen-presenting cells. The clones were specific for rPfCs and did not proliferate or secrete lymphokines when challenged with yeast-derived recombinant* P. vivax *or* P. berghei *CS protein or with a yeast-extract control. The clones also recognized the native CS protein in extracts of* P. falciparum, *but not* P. berghei *or* P. cynomolgi, *sporozoites.*

Introduction

Experimental rodent and primate hosts, including human volunteers, develop protective immunity after multiple exposures to the bites of irradiated malaria-infected mosquitos (*1*). We have recently examined the cell-mediated immune (CMI) responses of *Plasmodium falciparum* sporozoite-immunized rodent hosts and human volunteers using a recombinant *P. falciparum* circumsporozoite (rPfCS) protein.

The rPfCS was derived from yeast transformed with an expression plasmid (*2*) containing DNA of the T4 isolate of *P. falciparum* (*3*). The expressed recombinant protein contains approximately 70% (305/422 amino acids) of the total CS protein, including all of the repeats and the flanking regions 5′ and 3′ of the repeats ending at the first cysteine in the *C* terminal end of the CS protein.

The yeast-derived rPfCS protein was first heated and centrifuged to remove insoluble denatured protein, followed by purification to homogeneity using ion exchange and molecular sieve chromatography (*2*). Purity and integrity were demonstrated by SDS-PAGE, isoelectric focusing, amino-terminal sequence analysis, and immunoblotting with a monoclonal antibody to *P. falciparum* sporozoites.

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Methods and results

Murine humoral and CMI responses to yeast-derived recombinant P. falciparum *CS protein*

Studies on the immunogenicity of the rPfCS protein were carried out using nine congenic strains of mice. Following two injections of rPfCS, mice of all nine haplotypes developed antisporozoite antibodies detectable by indirect immunofluorescence (IFA) and radioimmunoassay (RIA). IgG IFA titres ranged from 200 to 1600 following the primary immunization. A secondary antibody response was detected following a booster inoculum of rPfCS, with all strains giving a similar high level of antisporozoite reactivity. RIA carried out with immune sera of each strain indicated that the anti-rPfCS antibodies were specific for the NANP repeat region of the *P. falciparum* CS protein.

Therefore, unlike the murine response to *P. falciparum* CS protein derived from vaccinia or baculovirus (*4*, *5*), the antibody response to yeast-derived recombinant CS was not genetically restricted. In order to determine whether this lack of genetic restriction was due to the presence of sporozoite specific,

rather than non-specific, T-helper cells in the rPfCS-immunized mice, we examined whether the rPfCS could specifically stimulate T-helper cells in *P. falciparum* sporozoite-primed mice.

Mice of two different haplotypes (H-2^k and H-2^d) were primed by inoculation of 10^5 *P. falciparum* sporozoites. The sporozoite-primed mice and control mice, which received normal salivary gland equivalents, were boosted with rPfCS/FCA (Freund's complete adjuvant). A second set of control mice received booster injections of adjuvant only. Sera, obtained three weeks after the administration of the rPfCS booster injection, were assayed using $(NANP)_3$ RIA.

The sporozoite-primed mice clearly produced an anamnestic antisporozoite antibody response following the inoculation of rPfCS. No increase in antisporozoite antibody titre was observed in the mice primed with normal salivary gland or in the adjuvant control mice. Therefore, sporozoite-induced memory T cells could recognize epitope(s) present on the rPfCS protein and function as helper cells in the antisporozoite antibody response.

The T-cell epitopes on the rPfCS were mapped using lymph node cells of rPfCS-immunized mice and overlapping synthetic peptides spanning the entire rPfCS protein (generously provided by M. Good). The immune lymph node cells proliferated in response to *in vitro* challenge with three peptides representing sequences in the *C* terminus of the CS protein. One of these peptides, aa 326–345, is a T-helper epitope (Th2R) previously defined using murine (*4*) and human (*6*, *7*) T cells. The other two peptides recognized by the cells of the rPfCS-immunized mice defined an epitope located 5′ to the Th2R epitope. This epitope, contained within the peptide sequence 301–320, has also been shown to stimulate proliferation of human PBL (peripheral blood lymphocytes) obtained from individuals living in Africa (*6*).

As has been found with the Th2R epitope, the amino acid sequence of this second epitope is polymorphic in the CS protein of different strains of *P. falciparum* (*8*). A single amino acid substitution has been reported in a Liberian *P. falciparum* strain (LE5), in which the alanine at position 314 is substituted by glycine. However, synthetic peptides containing either alanine or glycine at position 314 were recognized equally well by the rPfCS immune cells suggesting that these variant residues were not critical for antigen recognition.

CMI responses in P. falciparum *sporozoite-immunized volunteers*

In order to examine the human CMI response to sporozoites, studies were carried out using immune PBL obtained from *P. falciparum* sporozoite-immunized volunteers (See article by D. Herrington et al. on page 33). PBL of one sporozoite-immunized volunteer, exposed to 49 irradiated *P. falciparum* infected mosquitos, responded to *in vitro* challenge with rPfCS (SI 4.8). T-cell clones were obtained from this sporozoite-immunized donor by *in vitro* expansion of immune PBL using rPfCS and IL-2 followed by cloning by limiting dilution (*9*).

The T-cell clones proliferated in an antigen-specific and dose-dependent manner when challenged with rPfCS. Significant levels of ^{3}H-thymidine were incorporated when the clones were stimulated with minute amounts of rPfCS protein. No proliferative response was obtained in the absence of antigen-presenting cells (APC) or in the absence of antigen. Challenge of the T-cell clones with the same concentration of yeast-derived recombinant *P. vivax* or *P. berghei* CS protein did not stimulate the T-cell clones, nor did a yeast antigen control obtained from an equivalent amount of sham-transformed *Sacchromyces.*

All the clones were $CD4^+$ $CD8^-$ as determined by immunofluorescence using monoclonal antibodies. Consistent with the $CD4^+$ phenotype, the clones produced both IL-2 and gamma-interferon when stimulated by rPfCS. The amount of IL-2 detected in the cell supernatants, as measured by ^{3}H-thymidine incorporation by the Il-2-dependent murine CTLL line (*10*), correlated with antigen-induced T-cell proliferation.

Culture supernatants were also assayed for gamma-interferon using a commercial RIA (Centocor). Following challenge with 10 μg/ml of rPfCS, all the clones gave good proliferative responses. High levels of gamma-interferon were detected in the supernatants of the proliferating cells. In the absence of proliferation, i.e., following challenge with control antigens or medium, no gamma-interferon was detected.

The clones also recognized the native CS protein expressed by the parasite. All five clones proliferated and secreted lymphokine when challenged with extracts of *P. falciparum* sporozoites. The response to the sporozoite extract was species-specific. No proliferation, or gamma-interferon, was detected when the clones were challenged with comparable amounts of extracts of sporozoites of either *P. berghei* or *P. cynomolgi.*

In summary, a yeast-derived rPfCS protein has been used to measure both humoral and cell-mediated immune responses in *P. falciparum* sporozoite-immunized rodents and human volunteers. The murine T-cell epitopes mapped in the *C* terminus of the rPfCS protein and coincided with previously defined human T-cell epitopes. Studies are currently underway to examine the fine specificity of the human T-cell clones derived from a *P. falciparum* sporozoite-immunized volunteer.

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