Vaccine 32 (2014) 375–382

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Alterations in immunodominance of *Streptococcus mutans* AgI/II: Lessons learned from immunomodulatory antibodies

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ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form 22 October 2013
Accepted 6 November 2013
Available online 16 November 2013

Keywords:
Streptococcus
Monoclonal antibody
Immune complex
Immunodominance
Vaccine design

ABSTRACT

Streptococcus mutans antigen I/II (AgI/II) has been widely studied as a candidate vaccine antigen against human dental caries. In this report we follow up on prior studies that indicated that anti-AgI/II immunomodulatory monoclonal antibodies (MAbs) exerted their effects by destabilizing the native protein structure and exposing cryptic epitopes. We show here that similar results can be obtained by immunizing mice with truncated polypeptides out of the context of an intra-molecular interaction that occurs within the full-length molecule and that appears to dampen the functional response against at least two important target epitopes. Putative T cell epitopes that influenced antibody specificity were identified immediately upstream of the alanine-rich repeat domain. Adherence inhibiting antibodies could be induced against two discrete domains of the protein, one corresponding to the central portion of the molecule and the other corresponding to the C-terminus.

1. Introduction

Due to its etiological association with dental caries [1], multiple antigens of *Streptococcus mutans* have been studied as vaccine candidates [2–6]. One such protein is the cell-surface localized Antigen I/II adhesin [7], also called P1 [8], Antigen B [9], or PAc [10]. AgI/II family members mediate interactions with host salivary constituents, cell matrix proteins, and other bacteria (reviewed in [11]). Until recently, a lack of high-resolution structural information hindered the design and interpretation of immunological studies. As deduced from the primary sequence, AgI/II has discontinuous alanine (A)- and proline (P)-rich tandem repeats that flank a variable (V) region where strain differences are clustered [10,12,13]. Recently, an unusual tertiary structure was discovered in which the A-repeats form an α-helix that intertwines with the polyproline II (PPII) P-region helix to form a long narrow stalk [14]. The intervening segment including the V-region comprises a β sandwich arranged in two sheets [15]. The crystal structure of the C-terminus also revealed β sheet structure with three consecutive domains adopting a DE-variant IgG fold [16]. Hence, two globular regions lie on either end of an extended stalk. A high affinity intra-molecular interaction between the N-terminus, which has not been crystalized, and the C-terminus increases stability of AgI/II and enhances adhesive function [17]. The primary and modeled tertiary structures of AgI/II are illustrated (Fig. 1).

AgI/II's interaction with salivary components is complex and involves two distinct adherence sites [16,18]. The interaction differs depending on whether the major physiologic receptor, salivary agglutinin (SAG), is immobilized or is in fluid-phase. Monoclonal antibodies differ in their ability to inhibit adherence to SAG compared to SAG-mediated bacterial aggregation indicating that the determinants that mediate these two processes are not identical [19]. SAG is an oligomeric protein complex consisting primarily of the scavenger receptor glycoprotein gp340, and also containing amylase, sIgA and an 80 kDa protein [20,21]. Different regions of both gp340 [22] and AgI/II [19] contribute to the different interactions. *S. mutans* adherence *in vivo* involves binding of AgI/II to immobilized SAG within the salivary pellicle coating the tooth surface [23]. Disruption of this interaction by antibodies is the focus of preventative therapeutic protocols. In contrast, interaction of fluid-phase SAG with cell surface AgI/II represents an innate host defense mechanism [24,25], whereby aggregated bacteria are removed by swallowing. Hence it is desirable to elicit antibodies that disrupt SAG-mediated adherence, but not aggregation.

Numerous studies have demonstrated the relevance of an antibody response against AgI/II in protection against *S. mutans* colonization and cariogenicity (reviewed in [3,11,26,27]). Both salivary and serum antibodies, that enter the oral cavity *via* transudation through the gingival crevice, have been reported to be protective [6,28–33], or in some instances non-protective [34–36].

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http://dx.doi.org/10.1016/j.vaccine.2013.11.023

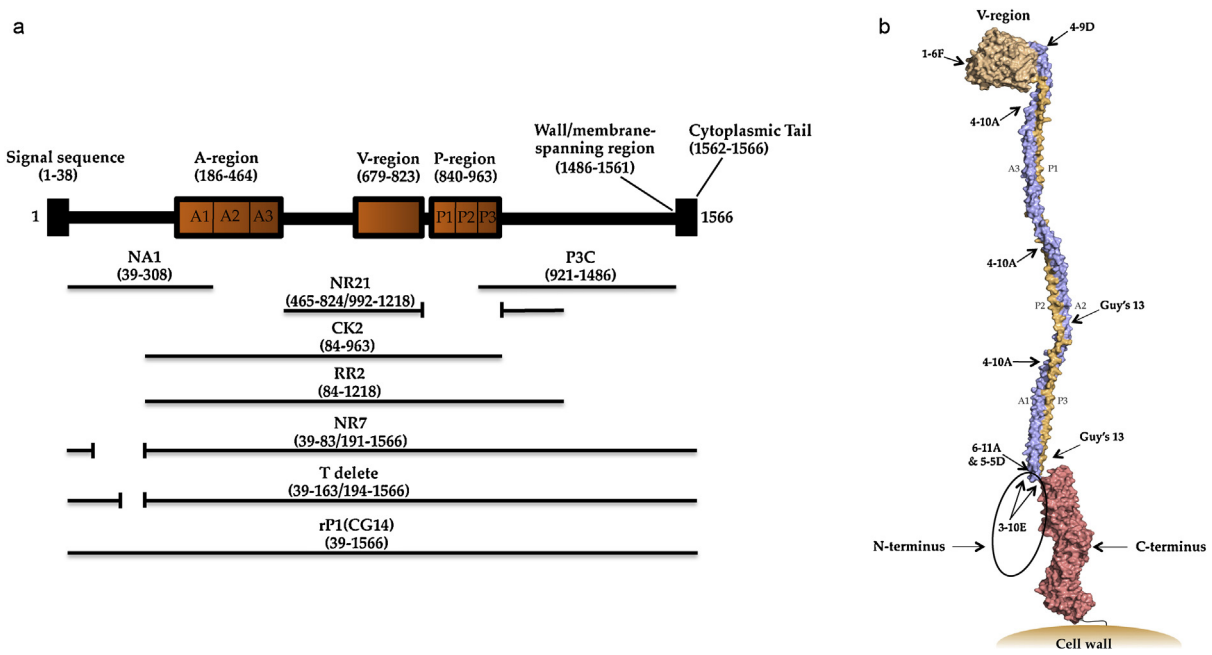

Fig. 1. Schematic representations of *S. mutans* Antigen I/II illustrating location of putative T cell epitopes and approximate antibody binding sites. (A) A representation of the primary structure of AgI/II and the recombinant polypeptides used in this study. (B) A three-dimensional model of Ag I/II. Approximate binding sites of monoclonal antibodies are indicated.

Subtle and potentially unapparent differences among immune responses can be crucial in determining the outcome of a host pathogen interaction. Naturally dominant epitopes are often not optimal for protection and pathogens can persist in the face of an immune response [37]. Therefore, it is fine specificity and functional activity, more so than total antibody amount, which likely determines whether colonization and cariogenicity is sufficiently inhibited to prevent disease by *S. mutans*.

Our laboratory has evaluated seven different anti-AI/II (P1) MAbs for immunomodulatory properties using an active immunization approach that incorporated them as part of immune complexes (IC) with whole bacterial cells [38–44]. Their approximate binding sites are illustrated in Fig. 1B and were deduced based on reactivity with internal deletion constructs and combinations of truncated polypeptides [44–47]. MAbs 1-6F and 4-9D are influenced by overall conformation and bind within the region intervening the A- and P-repeats. 4-10A recognizes a repeated epitope formed by interacting A- and P-region sequences. Guy's 13 also binds an epitope formed by interacting of A- and P-region sequences, but on a different part of the stalk. 6-11A, 5-5D, 3-10E bind epitopes that depend on the A-P interaction, but also involve a pre-A-post-P-region interaction. 3-10E's binding is almost completely eliminated when this interaction is disrupted. MAbs 1-6F, 4-9D, and 4-10A inhibit bacterial adherence, while 6-11A, 5-5D, 3-10E, and Guy's 13 do not [41,44].

Previous studies showed that when incorporated within ICs, MAbs 6-11A, 5-5D, 3-10E, 4-10A, and Guy's 13 redirected the adaptive immune response toward one of increased efficacy with regard to inhibition of bacterial adherence to SAG [38–44]. The presence of the MAbs within ICs altered the fine specificity and isotype composition of the elicited antibody response. These effects appeared to stem from a structural perturbation of the cell surface adhesin resulting in increased exposure of at least one normally cryptic or subdominant epitope, with the epitope recognized by 1-6F, an adherence-inhibiting MAb, shown to be affected [43,44]. In the current study we sought to determine whether the effects of anti-Ag I/II MAbs could be mimicked by immunization with truncated and internal deletion variants of AgI/II in which important target epitopes might be better exposed. As a result, novel putative T helper cell and C-terminal epitopes were identified.

2. Materials and methods

2.1. Bacterial strains, plasmids, expression and protein purification

S. mutans NG8 was grown aerobically for 16 h in Todd-Hewitt broth with 0.3% yeast extract (BBL, Cockeysville, MD). *Escherichia coli* strains were grown aerobically at 37 °C in Luria-Bertani broth (1% [wt/vol] tryptone, 0.5% [wt/vol] yeast extract, 1% [wt/vol] NaCl) supplemented with ampicillin (50–100 μg/mL) or kanamycin (25–50 μg/mL). Construction of the CK1 and RR2 [45], NA1, P3C, and NR7 [17], and NR21 [43] polypeptides has been described. Recombinant proteins were purified on amylose or nickel resin. An additional in-frame deletion construct of AgI/II lacking two putative T-cell epitope sequences (aa 164–193) was generated by circle PCR-mutagenesis using primers 5′-GCTGCTCATGAGGCAGCTGCAAATGCTGC and 5′-GCAGCATTTGCAGCTGCCTCATGAGCAGC with pCG14 [48] as template. Amplified DNA was self-ligated using Quick T4 ligase (New England Biolabs) and transformed into *E. coli* Top10. Plasmid DNA [17] with the confirmed deletion, pPC303, was transformed into *E. coli* M15 (pRep4). The histidine-tagged T-delete protein was purified on nickel resin following induction of mid-exponential phase cells with 1 mM IPTG for 2–4 h at 37 °C.

2.2. Mice

Six 8 week old female BALB/c mice were purchased from Charles River (Laboratories, Wilmington, MA) and housed in biosafety level 2 facilities under infectious disease conditions and fed a standard diet.