*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 2489–2493, March 1995
Microbiology

# Membrane cofactor protein (CD46) is a keratinocyte receptor for the M protein of the group A streptococcus

**(streptococcal infection/bacterial adherence/host–microbial interactions/virulence)**

NOBUHIKO OKADA*, M. KATHRYN LISZEWSKI†, JOHN P. ATKINSON*†, AND MICHAEL CAPARON*

Departments of *Molecular Microbiology and †Medicine, Washington University School of Medicine, St. Louis, MO 63110-1093

*Communicated by Emil R. Unanue, Washington University School of Medicine, St. Louis, MO, November 28, 1994*

**ABSTRACT** **The pathogenic Gram-positive bacterium *Streptococcus pyogenes* (group A streptococcus) is the causative agent of numerous suppurative diseases of human skin. The M protein of *S. pyogenes* mediates the adherence of the bacterium to keratinocytes, the most numerous cell type in the epidermis. In this study, we have constructed and analyzed a series of mutant M proteins and have shown that the C repeat domain of the M molecule is responsible for cell recognition. The binding of factor H, a serum regulator of complement activation, to the C repeat region of M protein blocked bacterial adherence. Factor H is a member of a large family of complement regulatory proteins that share a homologous structural motif termed the short consensus repeat. Membrane cofactor protein (MCP), or CD46, is a short consensus repeat-containing protein found on the surface of keratinocytes, and purified MCP could competitively inhibit the adherence of *S. pyogenes* to these cells. Furthermore, the M protein was found to bind directly to MCP, whereas mutant M proteins that lacked the C repeat domain did not bind MCP, suggesting that recognition of MCP plays an important role in the ability of the streptococcus to adhere to keratinocytes.**

The Gram-positive bacterium *Streptococcus pyogenes* (group A streptococcus) is an important human pathogen that causes a number of serious suppurative and inflammatory infections of the skin (impetigo, erysipelas, cellulitis, and necrotizing fasciitis) and of the throat (pharyngitis). The poststreptococcal sequelae that produce rheumatic fever and acute glomerulonephritis are of particular concern, as is the increasing incidence of invasive group A streptococcal infections, including the streptococcal toxic shock-like syndrome (1). Each streptococcal infection is initiated by the interaction of bacteria with host cells, and the consequence of this interaction induces a local inflammatory host reaction that finally forms suppurative lesions. This initial interaction between bacteria and host cells is mediated by the binding of structures on the bacterial surface (adhesins) to specific receptors on the surface of the host cells.

Epithelial cells of cutaneous tissue are target cells that *S. pyogenes* strains naturally infect and colonize (2). We have recently investigated the interactions between specific surface proteins of *S. pyogenes* and cutaneous tissue (3) and have shown that adherence of *S. pyogenes* to epidermal cells is mediated by two distinct surface proteins, protein F, an adhesin for respiratory epithelium (4, 5), and M protein, composed of two predominantly $\alpha$-helical protein chains arranged in a coiled-coil formation (reviewed in refs. 6 and 7). Further analyses revealed that M protein mediates the binding of streptococci to keratinocytes, while protein F directs binding to Langerhans cells located in the basal layer of the epidermis. It is interesting to note that keratinocytes, as initiators of cutaneous inflammation (8), and Langerhans cells, as antigen-presenting cells (9), both play important roles in host response to epidermal infection.

*S. pyogenes*, like most other Gram-positive bacteria, is a potent activator of the complement system (10). The M protein protects the organism from phagocytosis by the polymorphonuclear leukocytes that accumulate during the inflammatory response accompanying streptococcal infection (11). There is evidence suggesting that M protein exerts its antiphagocytic effect by altering the deposition of complement on the streptococcal surface (12). In addition, it has been shown that M protein selectively binds factor H to the surface of the bacterium where it can function as a cofactor for the inactivation of C3b by factor I (13) and potentially contribute to the inhibition of C3 deposition on the streptococcal surface.

In this study we have considered the possibility that a common mechanism underlies the factor H binding and adhesive properties of M protein. We determined that the C repeat domain of M protein proposed to bind to factor H also recognizes the keratinocyte receptor. Furthermore, membrane cofactor protein (MCP), or CD46 (15), a membrane-bound complement regulatory protein related to factor H (15), can serve as a cellular receptor for *S. pyogenes* on human keratinocytes.

## MATERIALS AND METHODS

**Bacterial Strains and Growth Conditions.** *Escherichia coli* DH5$\alpha$ (GIBCO/BRL) was the host for molecular cloning experiments and for expression of fusion proteins. *S. pyogenes* strains JRS4 (wild type) (16) and SAM1 (a protein F-deficient derivative of JRS4) (4) were used in adherence assays. *E. coli* was cultured in Luria–Bertani broth (17), and *S. pyogenes* strains were grown in Todd Hewitt medium (Difco) supplemented with 0.2% yeast extract (THY).

**Plasmid Construction.** Insertion of a 993-bp *Hpa* I–*Pvu* II fragment of pJRS42.50, which contains the complete gene that encodes the serotype 6 M protein (*emm6.1*) (18) into the *Sma* I site of the expression vector pQE30 (Qiagen, Chatsworth, CA) produced pNSP27 and generates a fusion protein with an N-terminal affinity tag consisting of six histidine residues (6×His). Deletion of a 398-bp internal *Hin*dIII fragment of pNSP27 generated pNSP28. Insertion of a 487-bp *Bam*HI–*Bsa*AI fragment of pNSP27 between the *Bam*HI and *Sma* I sites of pQE30 generated pNSP29, and deletion of a 302-bp internal *Kpn* I fragment of pNSP28 generated pNSP30. The structures of the affinity-tagged mutant M proteins are shown in Fig. 1. A specific domain of the MCP gene was amplified by PCR from a cDNA (clone K5-23) that encodes the MCP-BC1 isoform (20) using the following synthetic oligonucleotides; 5′-GCATGCTGTGAGGAGCCACCAACATTTG-3′ and 5′-GTCGACCAAACTGTCAAGTATTCCTTC-3′. The PCR product was cloned into pCRII (Invitrogen) to generate pMCP2.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MCP, membrane cofactor protein; SCR, short consensus repeat; MCPsol, soluble MCP; BSA, bovine serum albumin.

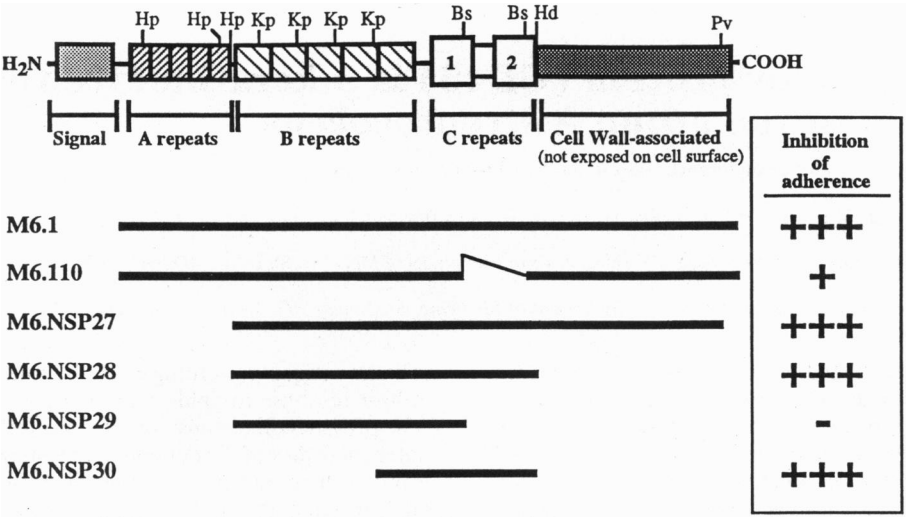


FIG. 1. The C repeat domain of the M protein recognizes a keratinocyte receptor. The structure of the M protein is illustrated along with the partial restriction sites of the *emm6.1* gene. Signal peptides (Signal), tandemly repeated regions (A, B, and C repeats), and the cell-wall associated region (which is the segment of molecule located within the cell wall) are shown. The full-length M protein (M6.1) and a series of mutant derivatives were used to examine the ability to inhibit bacterial adherence to epidermal keratinocytes in an *in situ* assay. The region of the internal in-frame deletion in M6.110 (19) is indicated by the thin line. The symbols indicate: −, no inhibition; +, <50% inhibition; +++, >80% inhibition, as determined by microscopic observations. Bs, *Bsa*AI; Hd, *Hin*dIII; Hp, *Hpa* I; Kp, *Kpn* I; Pv, *Pvu* II.

A 772-bp *Sac* I–*Stu* I fragment of pMCP2 was subcloned between the *Sac* I and *Sma* I sites of pQE30 to generate pMCP7.

**Purification of Fusion Proteins.** 6×His-tagged fusion proteins were purified from DH5α by nickel-nitriloacetic acid (Ni-NTA resin; Qiagen) affinity chromatography according to the recommendations of the manufacturer using native conditions for purification of the M protein derivatives and denaturing conditions for purification of the MCP fusion protein (MCP7). M protein derivatives were exhaustively dialyzed against a phosphate buffer (0.05 M, pH 7.8) that contained 0.3 M NaCl; and MCP7 was dialyzed against a Tris·HCl buffer (0.01 M, pH 6.3) that included 8 M urea and 0.1 M $NaH_2PO_4$. SDS/PAGE and immunoblot analyses confirmed that the appropriate fusion proteins were generated, and each preparation was >95% pure as estimated from Coomassie blue-stained gels.

**Streptococcal Adherence.** Analyses of adherence of streptococci to sections of normal human skin were performed as described and analyzed by staining with acridine orange and fluorescent microscopy (3). For adherence to cultured keratinocytes, the HaCat human keratinocyte cell line (21) was cultured in 24-well plates on glass coverslips and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum and 25 mM Hepes in a humidified atmosphere of 5% $CO_2$/95% air at 37°C for 3–4 days before use. Adherence of *S. pyogenes* to HaCat cells was assayed by the methods previously described and analyzed by Gram staining and light microscopy (3). For some experiments, the adherence of streptococci was quantified by enumeration of the number of cultured cells that bound bacteria, as described (3).

**Inhibition of Streptococcal Adherence to Human Skin Sections.** Deparaffinized and washed skin sections were incubated with each purified mutant M6 protein (50 μg/ml in PBS) or partially purified M6 protein (M6.1 and M6.110; 500 μg/ml in PBS) extracted from an *E. coli* periplasmic fraction (3, 22). This represents a concentration of between 1 and 5 mM for M6 and each mutant protein tested. After a 1-h incubation at room temperature, the sections were incubated with blocking buffer and then overlaid with the appropriate bacterial suspensions. In control experiments, periplasmic extracts from an *E. coli* strain that contained only the vector plasmid pUC18 had no inhibitory effect. Reduction in bacterial binding was estimated by enumeration of the number of adherent bacteria in two different microscope fields in two independent inhibition experiments.

**Pretreatment of *S. pyogenes* Strains with Factor H, Soluble MCP (MCPsol), and a Mutant MCP Derivative.** *S. pyogenes* strains were cultured overnight at 37°C in THY broth, harvested by centrifugation, and resuspended in PBS. Aliquots of bacterial suspensions were incubated with either factor H (Calbiochem), MCPsol (a gift of Cytomed, Boston), or a mutant MCP derivative (MCP7) (5–50 μg/ml for adherence to skin sections and 2–20 μg/ml for adherence to HaCat cells) for 1 h at room temperature with gentle agitation. Bacteria were centrifuged and washed once with PBS and resuspended in blocking buffer or DMEM. Control experiments showed that incubation of bacteria with PBS without the purified proteins (factor H and MCPsol) or with PBS containing 0.24 M urea without MCP7 had no effect on bacterial binding.

**Binding Assay.** Purified MCP7 was separated on SDS/PAGE, transferred onto a nitrocellulose membrane, incubated with blocking buffer containing 5% nonfat bovine milk and 0.05% Tween 20 in Tris-buffered saline (TBS; 0.05 M Tris·HCl/0.15 M NaCl, pH 7.4) at room temperature for 2 h and probed in TBS containing purified M6 protein derivatives (10 μg/ml) at room temperature for 4 h. The probed blots were washed three times with TBS containing 0.05% Tween 20 (TBS-T) and then incubated with a rabbit anti-M6 protein antiserum (1:500) overnight at room temperature. After washing three times with TBS-T, the blots were then incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin antiserum (1:500) (Dako) at 37°C for 2 h. The reaction was detected by using 4-chloro-1-naphthol as substrate (Sigma).

## RESULTS

**The C Repeat Region of M Protein Recognizes the Keratinocyte Receptor.** Recently, we have shown that the M protein of *S. pyogenes* is essential for the binding of the bacterium to keratinocytes in human cutaneous tissue (3). The structure of the surface-exposed region of the M molecule consists of three different domains of repeated sequence (A, B, and C repeats) (Fig. 1), and we constructed a series of mutant M protein derivatives and examined the ability of the purified proteins to inhibit the adherence of *S. pyogenes* to epidermal keratino-

Table 1. Inhibition of streptococcal adherence to keratinocytes by factor H and MCP

| Treatment* | Concentrations,† μg/ml | % inhibition of adherence‡ HaCat§ | % inhibition of adherence‡ *In situ*§ |
|---|---|---|---|
| PBS | — | 0 | 0 |
| BSA | 20/50 | 0 | 1 ± 8 |
| Factor H | 2/5 | 29 ± 5 | 32 ± 9 |
| | 20/50 | 56 ± 3 | 81 ± 5 |
| MCPsol | 2/5 | 31 ± 4 | 38 ± 6 |
| | 20/50 | 70 ± 5 | 77 ± 6 |
| PBS/urea | — | 2 ± 7 | 5 ± 8 |
| MCP7 | 2/5 | 41 ± 6 | 41 ± 9 |
| | 20/50 | 77 ± 4 | 86 ± 4 |

*Factor H and MCPsol were diluted in PBS. MCP7 was dissolved in PBS containing 0.24 M urea (PBS/urea).
†Proteins were tested at 2 and 20 μg/ml for HaCat cells and at 5 and 50 μg/ml in tissue sections.
‡The number of keratinocytes that bound streptococci in the presence of each inhibitor was determined. The data are expressed as the percentage of inhibition relative to the PBS control. Data represent the mean of duplicate experiments ± SEM (see *Materials and Methods* for more details).
§HaCat, cultured HaCat keratinocytes; *In situ*, keratinocytes in human skin sections.

cytes. As shown previously (3), full-length M protein (M6.1) inhibited the adherence of *S. pyogenes* to epidermal keratinocytes (Fig. 1). Similar inhibition of adherence was detected with deletion derivative M6.NSP27 (which lacks the A repeat region) and M6.NSP28 (a derivative of M6.NSP27 that lacks the cell wall region) (Fig. 1). However, a nearly full-length mutant M protein that lacks one of the two C repeats (M6.110) (19) could only partially inhibit streptococcal adherence (Fig. 1). The importance of the C repeats was further shown by the deletion of a region that encompasses almost the entire C repeat region from the inhibitory derivative M6.NSP28. The resulting mutant (M6.NSP29) was completely unable to inhibit adherence (Fig. 1). Finally, M6.NSP30 was an effective inhibitor of adherence and essentially consists of only the C repeat region (Fig. 1). From these results, we concluded that the C repeat domain is essential for the recognition of the keratinocyte receptor.

**Factor H Inhibits Streptococcal Adherence to Keratinocytes.** M protein has previously been shown to bind to factor H (13), a serum protein that inhibits activation of the complement cascade (15). Since it has been suggested that the C repeat domain of M molecule is responsible for the binding of factor H to M protein (14), we examined whether factor H could compete for recognition of the keratinocyte receptor. *S. pyogenes* JRS4 was incubated with purified factor H at room temperature for 1 h prior to overlaying on human skin sections. Efficient inhibition of bacterial adherence to epidermal keratinocytes was observed (Table 1 and Fig. 2). In contrast, no effect on adherence was observed by pretreatment of bacteria with bovine serum albumin (BSA) (Table 1).

Factor H belongs to a family of proteins that share in common a 60-amino-acid repeating motif termed the short consensus repeat (SCR) (15, 23). Because factor H is composed entirely of 20 SCRs, we speculated that the SCR motif is an important structure for recognition by M protein. Another member of the SCR family is MCP (CD46), a widely expressed membrane-bound complement regulatory protein known to be distributed on many epithelial cells including keratinocytes (24, 25). Like factor H, MCP also binds C3b and functions as a cofactor for the inactivation of C3b by factor I (15). The observation that the distribution of MCP, as detected by an indirect immunofluorescence method using a rabbit anti-MCP antiserum, paralleled that of adherent streptococci in cutaneous tissue (data not shown) suggested that MCP is an attractive candidate for a cellular receptor for *S. pyogenes*.

**MCP Serves as a Receptor for *S. pyogenes* on Human Keratinocytes.** To investigate a potential role for MCP, we tested whether purified derivatives of MCP could block strep-

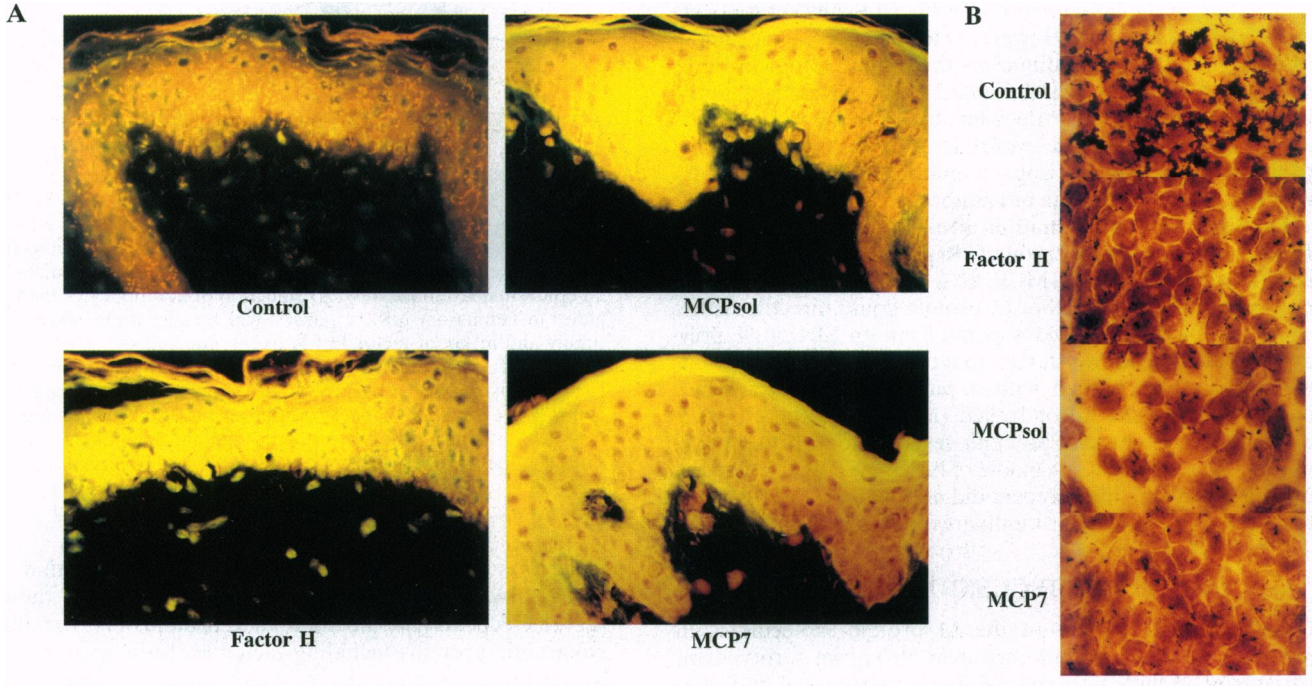


FIG. 2. Factor H and MCP inhibit streptococcal adherence to epidermal keratinocytes. The adherence of *S. pyogenes* JRS4 to human skin sections (*A*) and HaCat cells (*B*), which were preincubated without (Control) or with purified factor H, MCPsol, and MCP7 at concentration of 50 μg/ml for sections and 20 μg/ml for HaCat cells, is shown. (*A*) Adherent streptococci on the tissue sections, which were visualized by staining with acridine orange, appear as small orange-colored cocci. (×220.) (*B*) Streptococci bound on the surface of HaCat cells, which are visualized by staining with crystal violet, are small blue staining cocci. (×125.)

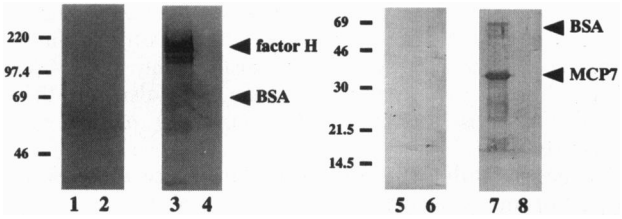


FIG. 3. Evidence for a functional molecular interaction between MCP and the C repeat domain of M protein. BSA (lanes 2, 4, 6, and 8), factor H (lanes 1 and 3), or MCP7 (lanes 5 and 7) was subjected to SDS/PAGE, transferred to nitrocellulose membranes, and then probed with either M6.NSP29 (lanes 1, 2, 5, and 6) or M6.NSP30 (lanes 3, 4, 7, and 8). Complex formation was identified using an anti-M6 antiserum, as described in *Materials and Methods.* In control experiments, the polyclonal anti-M6 antiserum recognized both M6.NSP29 and M6.NSP30. Arrowheads indicate the migration of factor H, MCP7, and BSA. Numbers represent the migration of protein standards of the indicated molecular masses (kDa). M6.NSP29, which lacks the C repeat domain, did not bind factor H (lane 1) or MCP7 (lane 6), whereas M6.NSP30, which contains this adherence-promoting region, bound both factor H (lane 3) and MCP7 (lane 7).

tococcal adherence. MCP is composed of four SCRs, a serine/threonine/proline-rich region, a 12-amino-acid area of undefined function, a transmembrane hydrophobic domain, a cytoplasmic anchor, and a cytoplasmic tail (for review, see ref. 20). We tested the ability of two different recombinant MCP derivatives, which contain only the surface-exposed regions of the MCP molecule, and found that both derivatives tested (MCPsol and MCP7) markedly reduced the adherence of JRS4 to keratinocytes in an *in situ* assay (Fig. 2*A*). Consistent with these results, MCPsol and MCP7 substantially inhibited binding to the HaCat human keratinocyte cell line (21) (Table 1 and Fig. 2*B*). Unlike the *in situ* assay, JRS4 showed some tendency to adhere as aggregates to HaCat cells. If MCP only blocked aggregate formation and did not block recognition of the keratinocyte receptor, no decrease in the number of keratinocytes that bound bacteria would be expected, since attachment of the initial streptococcal cell to any keratinocyte would not be inhibited. However, MCP was capable of inhibiting the number of keratinocytes that bound streptococci by 60–70% (MCP at 20 μg/ml; Table 1), demonstrating that the inhibitory effect of MCP does involve blocking receptor recognition. Identical results were obtained in the *in situ* assay (Table 1) and using the isogenic mutant strain SAM1 (4, 5), which expresses M protein but not protein F (data not shown).

**Evidence for the Formation of a Molecular Complex Between MCP and the M Protein C Repeat Domain.** To provide additional evidence for MCP as a cellular receptor for *S. pyogenes*, we tested whether M protein could directly bind to MCP. Purified MCP7 was separated on an SDS/10% polyacrylamide gel and transferred to a nitrocellulose membrane, which was then probed with a purified M protein which contained (M6.NSP30) or lacked (M6.NSP29) the C repeat domain. Consistent with previous results, the M protein containing the C repeat was capable of binding to MCP7, whereas M protein lacking the C repeat did not bind (Fig. 3). Identical results were obtained for binding to factor H (Fig. 3).

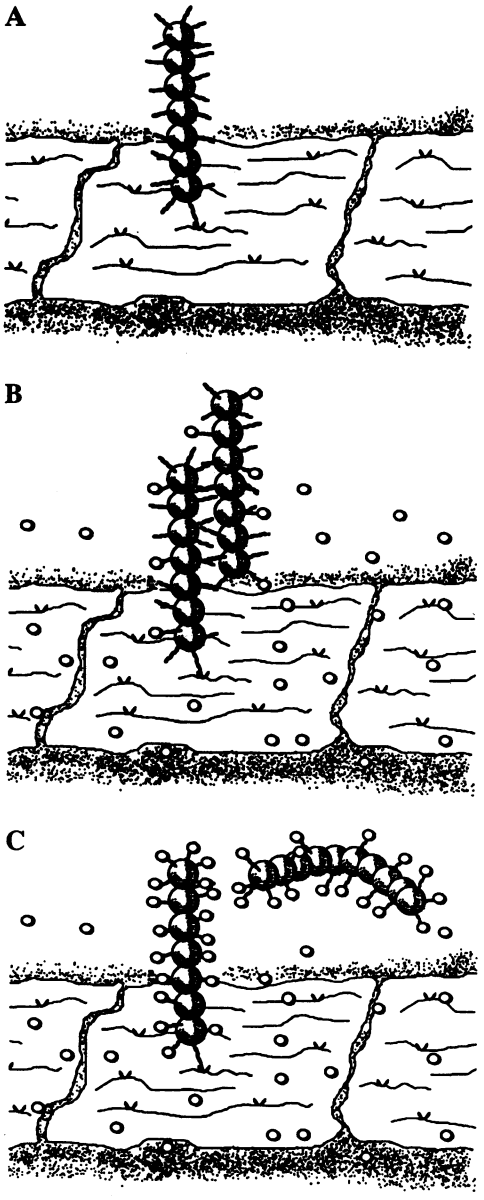


FIG. 4. Model for the interaction of M protein with MCP and factor H in streptococcal skin infections. (*A*) After entry into cutaneous tissue, *S. pyogenes* encounters environmental conditions favorable for the expression of M protein and consequently recognizes MCP on epidermal keratinocytes. (*B*) Infection of keratinocytes then stimulates inflammatory host responses, which cause an increased blood supply and influx of factor H (○) to the infected site. Upon contact with the inflammatory exudate, the M protein selectively binds factor H to the surface of the bacterium. (*C*) Streptococci that have bound factor H are no longer capable of interacting with keratinocytes. This may facilitate spreading streptococci to other locations of the host. Thus, competition between soluble factor H and cell-associated MCP for binding to M protein may function to control the adhesive potential of *S. pyogenes* during infection.

## DISCUSSION

One interesting feature of the M protein molecule is its antigenic diversity as there are over 80 known serotypeable variants of M molecules (6). While the N-terminal half of M protein contains the variable determinants of the M molecule, the C repeats share sequences highly conserved among different serotypes of M proteins (6). Therefore, it is not surprising that the C repeat region is critical for multiple M protein functions. Evidence that the C repeats can confer protection from experimental streptococcal infection in a mouse model has also suggested the importance of the conserved C repeats (27). In addition, M protein binds to multiple eukaryotic proteins including factor H, fibrinogen, filamentous actin (6), and human serum albumin (28). The C repeat region is also the binding domain for human albumin (28). Recent experiments utilizing a streptococcal mutant with single C repeat suggested that one is sufficient to protect the streptococcus from phagocytosis (19). In our experiments, however, deletion of a single C repeat resulted in a marked

decrease in ability to bind MCP, suggesting that the conservation of at least two copies of the C repeat in all streptococcal M proteins may be of selective advantage to *S. pyogenes* for the process of adherence and colonization on host cutaneous tissue.

In addition to complement regulatory proteins, SCR structures have been found in several other complement proteins that interact with C3b or C4b and complement-unrelated proteins, such as $\beta$ subunit of clotting factor XIII, $b_2$-glycoprotein I, interleukin 2 receptor, human haptoglobin, and coagulation factor C of the horseshoe crab (15, 22, 29). In some cases, the SCR is known to participate in protein–ligand interactions. However, in other cases, the significance of the SCR remains unknown. The fact that M protein can bind both factor H and MCP suggests that an epitope within the SCR sequence is important for M protein recognition. Furthermore, binding of an unglycosylated recombinant MCP to the C repeat domain of M protein suggests a protein–protein recognition.

Recently, it has been demonstrated that MCP serves as a receptor for measles virus (30–32). Identification and characterization of receptors for human pathogens have revealed that a number of organisms utilize the same eukaryotic surface proteins as their receptor. For example, cell surface proteins that bind to the eukaryotic glycoprotein fibronectin have been identified in a wide variety of bacteria that include streptococci, staphylococci, and mycobacterial species and *E. coli* (33). It is felt that the specificity of these interactions and the distribution of receptors on host cells are important determinants of a restricted range of hosts, tissues, and cell types that bacteria can utilize for colonization (26).

Based on the common mechanism that underlies the ability of M protein to interact with both factor H and MCP, we can propose a model for the role of streptococcal M protein in skin infections. Since streptococcal skin infections are generally initiated by the traumatic implantation of the organism from the surface of the skin, the interaction between MCP and M protein will allow the bacteria to bind to keratinocytes and serve to firmly establish the streptococcus at the initial focus of infection (Fig. 4*A*). However, the subsequent multiplication of the streptococcus in the tissue will damage the infected keratinocytes and stimulate the release of preformed interleukin 1$\alpha$ from these cells, which recruits both immune and inflammatory cells and triggers the release of a number of inflammatory mediators that induce increased vascular permeability and vasodilation (8). Since factor H is found in high concentrations in circulating blood (200–500 $\mu$g/ml), these inflammatory processes would result in a large influx of factor H to the infected site (Fig. 4*B*). Streptococci that bind factor H would no longer be capable of interacting with epidermal keratinocytes (Fig. 4*C*). This may facilitate spreading of bacteria throughout the tissue and contribute to the tendency of the streptococci to cause diffuse lesions in skin and to disseminate to deeper tissues.

We thank Vincent Fischetti for his gift of M6 antisera and Alice Pentland for her gift of HaCat cells. We also thank Jose Perez-Casal and June Scott for their interest in these studies and their helpful advice. N.O. and M.C. received support for this work from the Monsanto Co./Searle Biomedical Research Agreement Program. M.C. is an Established Investigator of the American Heart Association.

1. Stevens, D. L. (1992) *Clin. Infect. Dis.* **14,** 2–13.
2. Wannamaker, L. W. (1970) *N. Engl. J. Med.* **282,** 23–30.
3. Okada, N., Pentland, A. P., Falk, P. & Caparon, M. G. (1994) *J. Clin. Invest.* **94,** 965–977.
4. Hanski, E. & Caparon, M. G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6172–6176.
5. Hanski, E., Horwitz, P. A. & Caparon, M. G. (1992) *Infect. Immun.* **60,** 5119–5125.
6. Fischetti, V. A. (1989) *Clin. Microbiol. Rev.* **2,** 285–314.
7. Scott, J. R. (1990) in *Molecular Basis of Bacterial Pathogenesis*, eds. Iglewski, B. H. & Clark, V. L. (Academic, San Diego), pp. 177–204.
8. Kupper, T. S. (1990) *J. Invest. Dermatol.* **94,** 146S–150S.
9. Steinman, R. M. (1991) *Annu. Rev. Immunol.* **9,** 271–296.
10. Greenblatt, J., Boackle, R. J. & Achwab, J. H. (1978) *Infect. Immun.* **19,** 296–303.
11. Lancefield, R. C. (1962) *J. Immunol.* **89,** 307–313.
12. Weis, J. J., Law, S. K., Levine, R. P. & Cleary, P. P. (1985) *J. Immunol.* **134,** 500–505.
13. Horstmann, R. D., Sievertsen, H. J., Knobloch, J. & Fischetti, V. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1657–1661.
14. Fischetti, V. A. (1991) *Sci. Am.* **264,** 58–65.
15. Hourcade, D., Holers, V. M. & Atkinson, J. P. (1989) *Adv. Immunol.* **45,** 381–416.
16. Scott, J. R., Guenther, P. C., Malone, L. M. & Fischetti, V. A. (1986) *J. Exp. Med.* **164,** 1641–1651.
17. Scott, J. R. (1972) *Virology* **62,** 344–349.
18. Hollingshead, S. K., Fischetti, V. A. & Scott, J. R. (1986) *J. Biol. Chem.* **261,** 1677–1686.
19. Perez-Casal, J., Price, J. A., Maguin, E. & Scott, J. R. (1993) *Mol. Microbiol.* **8,** 809–819.
20. Liszewski, M. K., Post, T. W. & Atkinson, J. P. (1991) *Annu. Rev. Immunol.* **9,** 431–455.
21. Boukamp, P., Petrussevska, R. T., Breitkreutz, D., Hornung, J., Markham, A. & Fusenig, N. E. (1988) *J. Cell Biol.* **106,** 761–771.
22. Fischetti, V. A., Jones, K. F., Manjula, B. N. & Scott, J. R. (1984) *J. Exp. Med.* **159,** 1083–1095.
23. Reid, K. B. M. & Day, A. J. (1989) *Immunol. Today* **10,** 177–180.
24. Sayama, K., Shiraishi, S., Shirakata, Y., Kobayashi, Y., Seya, T. & Miki, Y. (1991) *J. Invest. Dermatol.* **97,** 722–724.
25. McNearney, T., Ballard, L., Seya, T. & Atkinson, J. P. (1989) *J. Clin. Invest.* **84,** 538–545.
26. Hultgren, S. J., Abraham, S., Caparon, M., Falk, P., St. Geme, J. W., III, & Normark, S. (1993) *Cell* **73,** 887–901.
27. Bronze, M. S., Courtney, H. S. & Dale, J. B. (1992) *J. Immunol.* **148,** 888–893.
28. Retnoningrum, D. S. & Cleary, P. P. (1994) *Infect. Immun.* **62,** 2387–2394.
29. Adams, E. M., Brown, M. C., Nunge, M., Krych, M. & Atkinson, J. P. (1991) *J. Immunol.* **147,** 3005–3011.
30. Manchester, M., Liszewski, M. K., Atkinson, J. P. & Aldostone, M. B. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2161–2165.
31. Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T. F., Rossi, B., Rabourdin-Combe, C. & Gerlier, D. (1993) *J. Virol.* **67,** 6025–6032.
32. Dörig, R. E., Marcil, A., Chopra, A. & Richardson, C. D. (1993) *Cell* **75,** 295–305.
33. Doig, P. & Trust, T. J. (1993) *J. Microbiol. Methods* **18,** 167–180.