

Detection of Streptococcal Class I M Protein in Psoriasis by Confocal Immunofluorescent Microscopy

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Epidemiological evidence implicates *Streptococcus pyogenes* (group A) infection as a common triggering stimulus for psoriasis. Unequivocal demonstration of streptococcal antigens in psoriatic skin has been difficult due to cross-reactive antigens in both normal human tissue and group A streptococci, which complicate immunohistological analysis. In this study cryostat sections of involved psoriatic skin were stained with monoclonal antibody 111–15504 to group A streptococci. The epitope recognized by this antibody was found to be specific for group A streptococci and is associated with class I M protein.

Streptococcal antigens were found in the dermal papillae and epidermis of psoriatic skin lesions of 20 out of 38 patients. These findings indicate that specific *S. pyogenes* antigen, associated with class I M protein, is often present in psoriatic lesions. Such an antigen, originating from focal infection elsewhere could be responsible for T-lymphocyte inflammatory responses triggering the development of psoriatic lesions. **Key words: focal infection; *Streptococcus pyogenes* antigen/antibody.**

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The association between β -hemolytic (group A) streptococci and psoriasis has long been recognized (1). It is known that guttate psoriasis correlates with throat infections and raised antibody titers to streptococcal antigens (2, 3). Furthermore, prophylactic penicillin often inhibits recrudescence of the disease (4). Chronic plaque psoriasis can also exacerbate during convalescence from acute streptococcal pharyngitis (5). Unequivocal demonstration of streptococcal antigens in psoriatic lesions has not been successful. Indeed, results of immunohistological analyses have been difficult to interpret, since cross-reactive antigens are present in both normal human tissue and group A streptococci (6, 7). In an attempt to overcome this difficulty we screened several commercially available monoclonal (mAb) and polyclonal antibodies to group A streptococci and their products, to identify those with specificity for bacterial antigens but non-reactive with normal skin tissue (8). One anti-group A streptococcal mAb, 111–15504, which stained group A streptococci, showed no detectable reactivity within normal human skin, and was used to stain skin biopsies of psoriatic skin.

MATERIALS AND METHODS

Thirty-eight patients with psoriasis were examined (Table I). Control groups included 5 healthy volunteers with normal skin and 11 patients with skin diseases other than psoriasis: lichen planus [2], pityriasis

rosea [2], cellulitis [1], warts [6], hemangioma [1] and herpes simplex [1].

Detection of streptococcal antigens in psoriatic skin

Skin samples. Skin specimens were obtained by means of transverse upper level excision biopsy with Gillette "Blue blade", performed under local anesthesia with 1% lidocaine (9). In patients with psoriasis skin samples were taken from both lesional and non-involved areas. In control patients with non-psoriatic skin diseases samples were taken from involved areas. In healthy control subjects skin biopsy specimens were obtained from a variety of sites.

Indirect immunofluorescence assay (IFA). Fresh skin specimens were embedded in OCT compound and sectioned at -20°C . Six- μm horizontal cross-sections were processed for immunofluorescence staining according to a standard protocol (8). MAb 111–15504 to *S. pyogenes* (working dilution 1:40) (O.E.M. Concepts, Toms River, New Jersey, U.S.A.) was used as the primary antibody, and sheep anti-mouse IgG antibody conjugated with fluorescein isothiocyanate (FITC) (Sigma Chemical Company, St Louis, MO, U.S.A.) (working dilution 1:100) was used as the second or reporter antibody. Control samples were included, in which either the primary mAb was omitted or replaced with non-immune mouse serum. No immunofluorescent staining was observed in any of the control samples.

Microscopy. Specimens were mounted with glycerol mounting medium and examined in a Nikon "Labophot" epifluorescence microscope with a mercury lamp, FITC exciter and 525 nm barrier filter. The same slides were also studied in MRC-600 Series Laser Scanning Confocal Imaging System with 15 mW Krypton/Argon mixed gas multi-line laser.

Comparative immunochemical analysis of mAb 111–15504

Reaction with reference group A serotyping streptococcal isolates. Isolates of group A streptococci, representing the various M serotypes, obtained from The Rockefeller University Culture Collection (Table II), were grown as stationary cultures at 37°C for 24 h in Todd Hewith broth (BBL, Gaithersburg, MD, U.S.A.). Cultured microorganisms were harvested by centrifugation, washed and resuspended in normal saline at a concentration of $10^8/\text{ml}$. Ten μl of each suspension were placed on a slide, air-dried and stained with mAb 111–15504 or an irrelevant antibody, as described earlier (Fig. 1A).

Reaction with recombinant M gene family proteins. Previous studies by Raeder et al. (10) have demonstrated that treatment of group A streptococci with CNBr in HCl is the optimal method for solubilizing the anti-phagocytic M protein. This procedure resulted in extraction of a limited number of polypeptides, all of which are derived from proteins belonging to the M or M-related gene family. This procedure, coupled with monoclonal or polyclonal antibodies, has enabled these proteins to be classified into one of two major antigenic classes (11).

Bacterial proteins solubilized from group A streptococci were separated in 10% SDS polyacrylamide gels (10). Separate proteins were transferred to nitrocellulose by electroblotting and probed with specific antibodies to streptococcal proteins. Resulting antigen antibody complexes were detected as described previously (10).

Antibodies to group A streptococci. Three antibodies were analyzed:

- (1) Polyclonal antibody to a representative class I M protein (Emm55) was generated in chickens, as described previously (12). This antibody, designated anti-type IIo, has been previously shown to

Table I. Skin tissue staining with mAb 111-15504 to *S. pyogenes*

Specimen source	Number of positively stained/number of patients ^a	Pattern of positively stained samples			
		Papillar	Basal layer	Diffuse epidermal	Scattered cells
Psoriasis ^b					
Guttate	6/14	3	3	2	3
Plaque	12/22	10	3	5	2
Pustular	2/2	1	0	1	1
Healthy control	0/5	0	0	0	0
Non-psoriatic skin diseases	1 ^c /13	0	0	0	0

Note: ^a Out of 11 patients who had documented evidence of focal infection, streptococcal antigen was present in the skin samples of 7 (63%).

In all 20 positively stained patients immunofluorescence was observed only in biopsy specimens from lesional skin, non-involved skin biopsy samples from the same patients did not demonstrate any fluorescent staining.

^b Guttate psoriasis is defined as an outbreak of discrete small (<0.5 cm diam) scaly papular skin lesions; plaque psoriasis—chronic coin-size lesions (>0.5 cm diam); pustular psoriasis—numerous superficial pustules.

^c Immunofluorescent staining of *S. pyogenes* was observed in a patient with cellulitis (8).

Table II. Reaction of anti-streptococcal 111-15504 mAb with different serotypes of *S. pyogenes*

<i>Streptococcus pyogenes</i>	Positive staining	Negative staining
Class I ^a	Type 1 T1/195/2 Type 3 B930/61/3 Type 6 S43/192/1 Type 8 C265/86/1 Type 12 T12/126/4 Type 13 T13/150/2 Type 18 J17C55/2 Type 55 A928 Type 57 A995 MNT T6/14/17/23/47	Type 14 T14/46/7 Type 17 J17E/135/3 Type 30 D24/126/2
Class II		Type 2 T2/44/RB4/119 Type 4 T4/95/RB5 Type 9 T9/120/1 Type 11 T11/81/4 Type 22 T22/76/3 Type 49 B737/71/2

^a Classification of *S. pyogenes* serotypes is based on data from Boyle & Raeder (11) and Bessen et al. (13).

identify class I M proteins in an immunologically specific manner (12).

- (2) The mAb 10B6, first described for its specificity towards an epitope present only in class I M proteins (13), was a gift from Dr. Vincent Fischetti, Rockefeller University, New York, New York.
- (3) The mAb 111-15504 (O.E.M. Concepts, Toms River, New Jersey, U.S.A.) was known to recognize an epitope associated with group A streptococci; however, the specific epitope recognized by this reagent has not been identified prior to this study.

Recombinant M proteins. Representative class I and class II M proteins cloned from group A streptococci were utilized for studies designed to establish the specificity of mAb 111-15504. These included Emm49 (14), Emm55 (12), Emm64/14 (15) and Emm1 (16). The cloning isolation and characteristics of these recombinant proteins have been described previously (12, 14, 15).

IFA with mAb 10B6. The specimens from 6 patients that had demonstrated positive staining with mAb 111-15504 were stained with mAb 10B6. This antibody had been previously characterized and

shown to recognize a unique epitope expressed on class I streptococcal M proteins (17). Staining was performed as described earlier for mAb 111-15504 with the exception that mAb 10B6 or an isotype control matched mAb with an irrelevant specificity was substituted for mAb 111-15504.

RESULTS

In preliminary screening studies mAb 111-15504 was found to recognize an epitope on the surface of certain group A streptococcal isolates, while failing to recognize any epitopes present in normal human skin. This antibody was used to screen for the presence of streptococcal antigens in lesional psoriatic skin samples.

The results presented in Table I demonstrated positive immunofluorescence in 20 out of 38 patients studied. None of the samples demonstrated fluorescent staining if the primary antibody was omitted or if a non-immune mouse serum was substituted. Qualitative and quantitative variation among positive samples was observed (Fig. 1B-F). Streptococcal antigen was most frequently found inside dermal papillae around capillaries, and inside the cells of the epidermal basal layer. In some cases diffuse epidermal staining was observed. A few patients demonstrated particulate immunofluorescence of scattered epidermal cells. Cells stained in a particulate manner were found sometimes inside hair follicles. Immunofluorescent staining of epidermal dendritic cells was also observed in several biopsy samples.

Stratum corneum staining was variable and non-specific and hence was disregarded.

In patients with psoriasis immunofluorescence was observed only in the samples taken from the lesional skin. Samples obtained from non-involved areas did not demonstrate any fluorescent staining.

Specimens of normal control skin and non-psoriatic lesional skin, with the exception of the patient with cellulitis, did not demonstrate any fluorescence when stained with mAb 111-15504.

These results suggest that mAb 111-15504 identified a unique epitope associated with a streptococcal product that is not present in normal human skin tissue. In order to identify the streptococcal epitope recognized by this antibody, a series of immunochemical studies were performed. In the initial

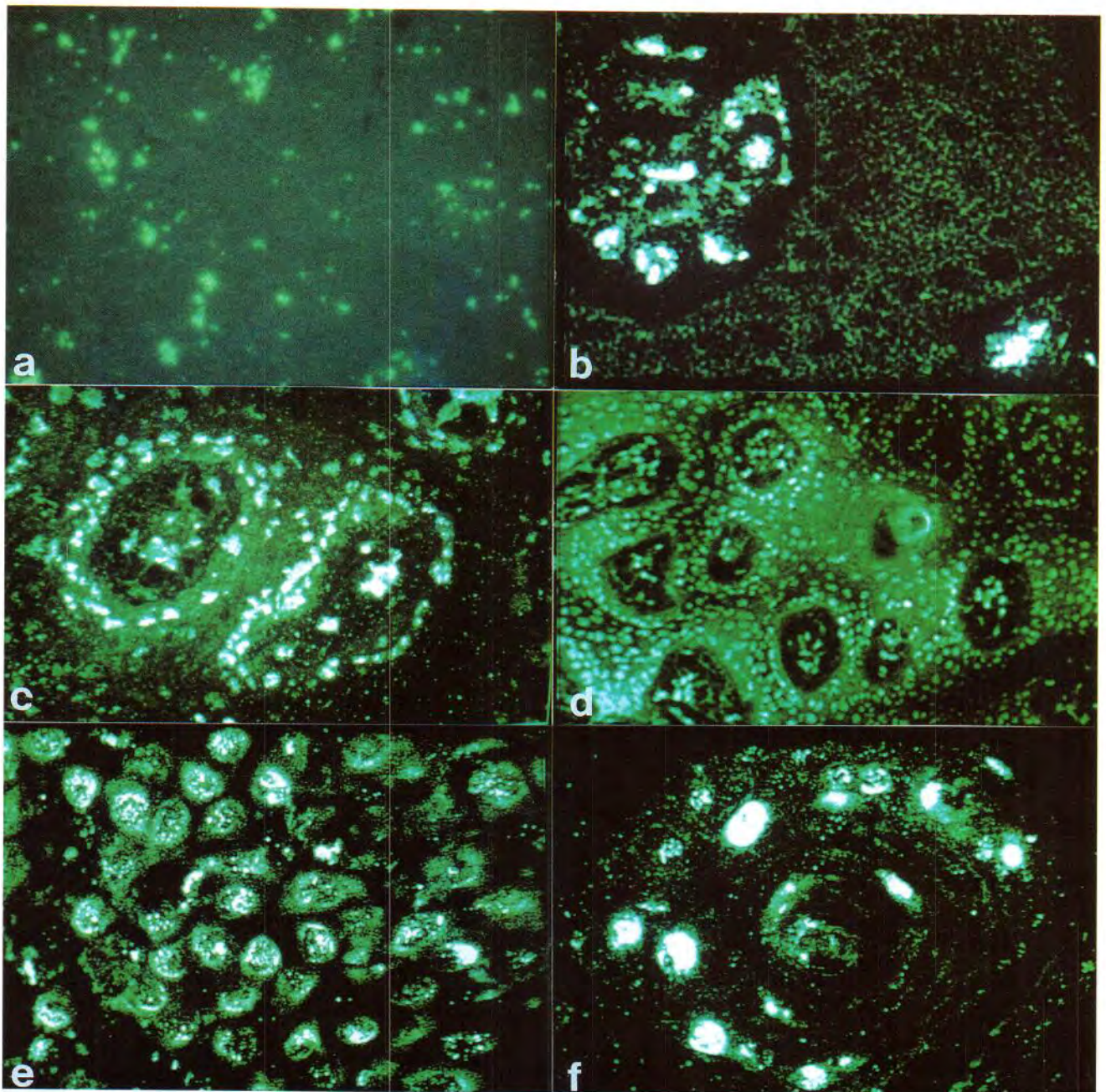


Fig. 1. (a) Immunofluorescence staining of *S. pyogenes* isolate MNT T6/14/17/23/47 with mAb 111-15504 ($\times 400$). (b-f) Demonstration of *S. pyogenes* antigens in horizontal cross-sections of psoriatic lesions from human skin by immunofluorescence. Representative sections viewed, by confocal microscopy using mAb 111-15504 to specifically identify streptococcal antigens present in the human skin sections. (b) Positive immunofluorescence staining inside dermal papillae ($\times 600$). (c) Streptococcal antigen localized in papillae and basal layer cells ($\times 400$). (d) Diffuse epidermal staining and antigen deposition inside papillae ($\times 200$). (e) Specific particulate immunofluorescence of scattered epidermal cells ($\times 600$). (f) Streptococcal antigens inside hair follicles ($\times 600$).

experiments, immunofluorescent analysis was carried out with a variety of different reference group A serotyping isolates. The results presented in Table II demonstrate that these organisms could be divided into two groups based on their reactivity with mAb 111-15504. Previous studies by Bessen and colleagues (13) had identified two major lineages of group A streptococci that could be differentiated based on the antigenic class of M protein expressed. The studies reported in Table II demonstrate a clear association between isolates

reactive with mAb 111-15504 and those capable of expressing class I M proteins.

Based on this observation, further analysis was carried out to determine if mAb 111-15504 was recognizing an epitope associated with a class I M protein. In preliminary studies, a series of well characterized recombinant class I and class II M proteins were tested for reactivity with antibody 111-15504 in a dot blot assay procedure. The results obtained were consistent with the prediction that this antibody



Fig. 2. Western immunoblot analysis of the specificity of mAb 111-15504. CNBr extracts of two representative isolates expressing class I M proteins and one representative isolate expressing class II M proteins were separated in 10% SDS polyacrylamide gels under reducing conditions. The separated proteins were transferred to nitrocellulose, and parallel strips were probed with either a biotinylated chicken anti-type IIo antibody followed by streptavidin enzyme reporter system (lane 1); a mAb 10B6 followed by a goat anti-mouse second antibody reporter system (lane 2); or mAb 111-15504 followed by a goat anti-mouse second antibody reporter system (lane 3). Panel A contains extracts from an M1 serotype isolate which expresses an M1 ~50,000 class I M protein. Panel B contains extracts from an M49 serotype isolate which expresses an M49 ~48,000 class II M protein. Panel C contains extracts from an M non-typeable isolate, 64/14, that expresses an M ~55,000 class I M protein. For precise experimental details, see Methods.

recognized specifically an epitope present in class I M proteins (data not shown).

Previous studies by Bessen and colleagues had identified a unique epitope present on class I M proteins that was recognized by the mAb 10B6 (13). Consequently, in the next experiment the polyclonal anti-type IIo, the mAb 10B6 and the mAb 111-15504 were compared by western immunoblotting techniques for their ability to identify a class I M protein present in the CNBr extract of representative group A streptococcal isolates. The results of this analysis demonstrated that all three antibodies recognized a singular protein band, which migrated with the expected Mr for the class I M protein of each isolate (Fig. 2). None of these antibodies recognize any epitopes present in a CNBr extract containing a representative class II M proteins. In view of these results, it is probable that this antibody recognizes an identical or closely related epitope to that recognized by mAb 10B6.

To test our prediction that mAb 111-15504 and mAb 10B6 recognize a similar epitope, a series of biopsied sections from psoriatic lesions was stained with mAb 10B6. The results, using mAb 10B6 as the primary antibody, (presented in Fig. 3), demonstrate a pattern of selective staining similar to that obtained with mAb 111-15504. The 10B6 reagent failed to recognize any antigens present in normal human skin tissue. These results further support the hypothesis that an epitope present in M proteins of antigenic class I is being detected in psoriatic lesions.

DISCUSSION

Psoriasis is an inflammatory skin disorder, possibly induced by antigens, as yet unidentified, which trigger T-lymphocytes to induce marked proliferation of the epidermis (18). Epidemiological evidence implicates *S. pyogenes* (group A) as a common triggering stimulus for psoriasis. Onset of the disease frequently follows a streptococcal throat infection, and serum from psoriatic patients often shows elevated anti-streptococcal antibody titers (19, 20). Psoriatic lesions have

also been produced at injection sites of streptococcal antigen in the skin of psoriatic patients (21). T-cells specific for group A streptococcal antigens can be isolated from the skin lesions of guttate psoriasis (22). Since psoriatic skin lesions are sterile, it is believed that streptococcal antigens, whether nominal or superantigens, elicit an immune response targeting a cross-reactive epitope present on epidermal tissue (6, 7, 23, 24).

The existence of epitopes on streptococcal proteins that cross-react with epitopes on normal tissue has made it difficult to demonstrate the presence and persistence of streptococcal antigens in psoriatic lesions. In this study positive staining was observed in psoriatic lesions stained with mAb 111-15504, while no staining was observed in control skin biopsy specimens from non-involved areas, normal skin or other skin disease lesions. When an isotype-matched mAb of irrelevant specificity was substituted, no reactivity was observed in either psoriatic lesions or healthy skin. This supports the conclusion that a specific epitope, associated with a streptococcal antigen, was detected in psoriatic lesions.

The precise epitope recognized by mAb 111-15504 has not been previously defined. Our study showed that mAb 111-15504 specifically recognized an epitope associated with class I M proteins. Previous studies by Bessen et al. had defined two major lineages of group A streptococci, based on the antigenic properties of their M protein (13). A mAb 10B6 was defined as capable of distinguishing a unique epitope present in M proteins of antigenic class I and absent in class II M proteins (17). A comparison of the specificity reported for antibody 10B6 and observed in our studies for 111-15504 suggested that both mAbs recognize an identical or closely related epitope on class I M proteins. The epitope recognized by mAb 10B6 antibody has been mapped to a region in the center of class I M proteins (17). This epitope maps to a different region of M proteins from the epitope responsible for eliciting antibodies that cross-react with normal human tissue (25). The pattern of staining obtained using mAb 10B6 and mAb 111-15504 was similar, indicating that an epitope associated with class I M proteins was being detected in psoriatic lesions from over 50% of patients studied.

Antigenic class I M proteins were expressed by many commonly isolated streptococci from patients with respiratory tract infections (26). Class I M proteins can bind to specific human skin cells (27, 28) as well as to an array of human plasma proteins (11). Circulating immune complexes containing class I M proteins originated from focal infection areas conceivably could be deposited or trapped at sites of capillary injury (3, 29), as evidenced by their presence around skin capillaries and in adjacent epidermal cells of psoriatic lesions. Cells found along the dermo-epidermal junction in psoriasis may act as antigen-presenting cells (30).

It has been shown recently that group A streptococci, although regarded typically as extracellular pathogens, can be internalized *in vitro* by epithelial cells (31). Experimental models have also shown that certain streptococcal antigens are not effectively cleared from the host and can persist in the skin for a long time, causing relapsing chronic inflammation (32).

Numerous studies suggest that M proteins consist of several defined domains that account for their various roles in adherence, invasiveness (33) and antiphagocytic activity (34), properties that contribute to the overall virulence patterns of

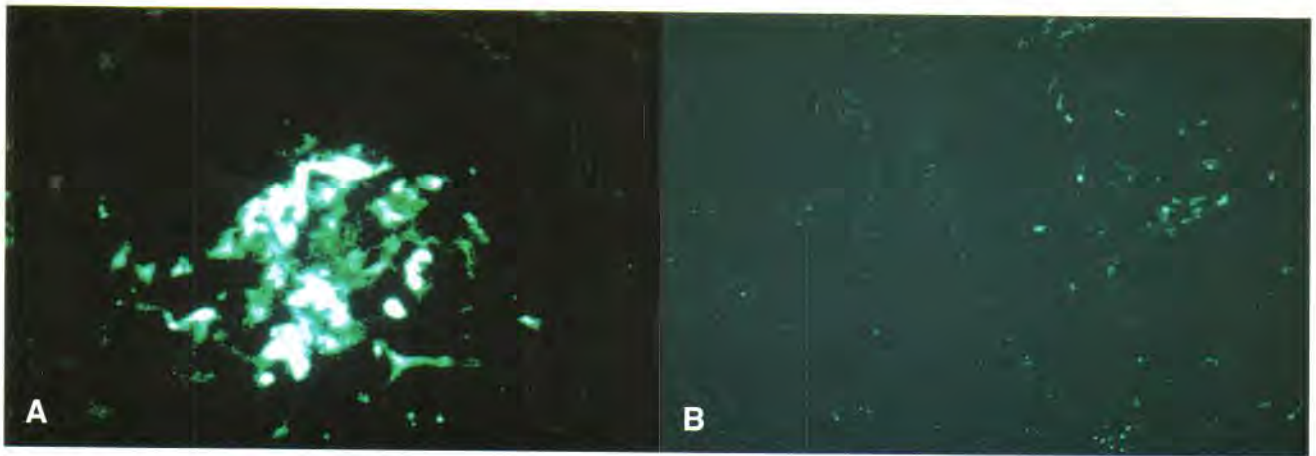


Fig. 3. Confocal microscopy of horizontal cross-sections of psoriatic skin lesions stained with either mAb 10B6, which is specific to class I M protein of *S. pyogenes* (A), or an isotype control matched mAb specific to hen egg lysozyme (B) ($\times 800$). Streptococcal antigens were found to be deposited inside dermal papillae upon staining with mAb 10B6 but not with a control mAb.

group A streptococci. Some epitopes on these proteins are cross-reactive with human tissue (35).

The presence in a single protein of a unique bacterial epitope as well as epitopes expressed by human tissue might elicit a mixed immunologic response, including an anti-bacterial response to the foreign epitope in addition to a concomitant autoimmune response to the self-like epitope (24, 35). This combined response might target a single bacterial antigen which, as our study demonstrates, can persist in the skin of patients with psoriasis.

While this study identifies the class I M protein as a potential trigger and target for immune reactions in the skin, it is clearly not the only etiologic agent contributing to psoriasis. Other streptococcal antigens and antigens from other bacteria, fungi and viruses might also trigger this condition (36–38). Recognition of a bacterial antigen which contains both foreign epitopes and epitopes with cross-reactive structures to normal tissue may help account for the pathological characteristics of psoriasis in genetically susceptible individuals.

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