13-*cis*-retinoic acid did not affect PGE_2 production by rat synovial cells in vitro. However, we do not know whether 13-*cis*-retinoic acid affected the production of PGE_2 by other cell types in vivo and thereby influenced the development of cell-mediated immunity in the rats with AA.

Retinoids modulate the effects of other mediators. For example, the action of mononuclear cell factor (probably interleukin-1), which stimulates the production of collagenase and PGE_2 by synovial cells in culture, is antagonized by 13-*cis*-retinoic acid (*5*). Retinoids also antagonize the proliferative effect of prolactin on mammary epithelium (*19*) and prevent polypeptide growth factors from inducing expression of phenotypic characteristics of transformed cells in nonneoplastic cells (*20*).

Trentham and Brinckerhoff (*21*) recently have studied the effect of treatment with 13-*cis*-retinoic acid on the development of collagen-induced arthritis in rats. In this model, the administration of retinoic acid was associated with an increase in the severity of the arthritis with no effect on humoral or cellular immune responses to collagen. Synovial cells taken from rats with collagen-induced arthritis also secreted elevated concentrations of collagenase and PGE_2. Oral administration of 13-*cis*-retinoic acid decreased collagenase production by these synovial cells. Thus, despite the fact that 13-*cis*-retinoic acid affected AA and collagen-induced arthritis differently, its effect on collagenase production by synovial cells was the same in both models.

Reasons for the disparate effects of treatment with 13-*cis*-retinoic acid on the severity of arthritis in the two models are unclear, but the dissimilarities may be due to differences in (i) the sex of the rats used (females for collagen arthritis and males for AA), (ii) the dosage of retinoid (rats with collagen arthritis received in the diet only a quarter of the amount of drug administered to rats with AA), or (iii) mediators of the two models of the disease. Other recent experiments have demonstrated that all-*trans*-retinoic acid and corticosteroids at low concentrations ($10^{-10}M$) synergistically inhibit collagenase production by synovial cells in culture (*15*).

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Expression of Streptococcal M Protein in *Escherichia coli*

Abstract. *The structural gene for group A streptococcal M protein, the fibrillar surface molecule enabling the organism to resist phagocytosis, has been cloned into* Escherichia coli. *The molecule produced by* Escherichia coli *is slightly larger than the M protein isolated by solubilization of the streptococcal cell wall, but is similar in size to that secreted by streptococcal protoplast and L forms. Immunologically, the molecule synthesized by* Escherichia coli *has the same type-specific determinants as the streptococcal M protein.*

In developing countries of the tropics and subtropics, rheumatic heart disease is both the major cause of cardiac hospital admissions and the most common form of cardiac damage in school-age children (*1, 2*). Although the exact mechanisms of disease causation are not well understood, it is clear that rheumatic fever, as well as acute nephritis, follows infection with *Streptococcus pyogenes* (group A streptococcus). Fortunately, since group A streptococci are all sensitive to penicillin at this time, prompt treatment with this antibiotic is effective in preventing acute rheumatic fever (*3*). However, for those poorer countries in which these diseases are prevalent, such therapy is both expensive and logistically impractical. Furthermore, it is possible that penicillin-resistant streptococci may arise at any time. For these reasons, the development of a safe and effective vaccine to prevent infection by the group A streptococcus is a desirable objective.

Resistance to group A streptococcal infection depends on the presence of type-specific antibodies to the M protein (*4*), a fibrillar molecule found on the surface of the organism (*5, 6*). The M protein is a major virulence factor of this bacterium because it can impart resistance to attack by the host's phagocytic cells (*7*). Because of its ability to vary the antigenic structure of the M molecule, the group A streptococcus is able to successfully evade the host's type-specific immune response and thus cause recurrent streptococcal disease in man. In addition to a number of nontypable strains, about 70 immunologically distinct M types are now recognized. Despite the fact that antibodies cross-reac-

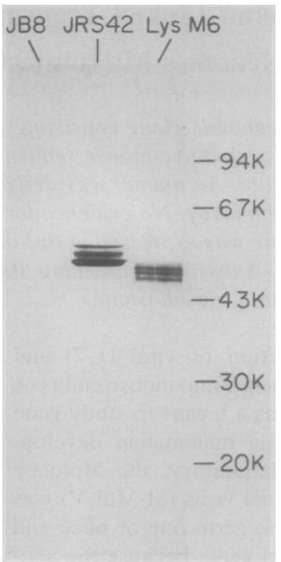
tive among certain M types are common, only antibodies prepared against the homologous type are capable of initiating phagocytosis (*8–10*).

Understanding of the relation of the structure of the different M proteins to their function may lead to the development of an effective antistreptococcal vaccine. To begin a molecular characterization of the M protein, we have used in vitro DNA recombinant technology to clone its structural gene into *Escherichia coli*.

For the cloning, a cosmid vector requiring very large DNA insertions was used to minimize the number of colonies to be screened. In the 5.4-kb vector pJB8 (*11*), the entire streptococcal genome should be contained in about 200 *E. coli* transductants. The vector DNA was digested with Bam HI, which cleaves pJB8 at a single site, and was treated with calf intestinal alkaline phosphatase to prevent religation of vector ends with each other. Streptococcal phage lysin was used to lyse the bacteria (*12*), and DNA was then isolated from the M6 *Streptococcus pyogenes* strain D471 and partially digested with Sau 3a, which recognizes a four base-pair (bp) sequence and thus should give an essentially random collection of DNA fragments. The vector and insert DNA preparations were ligated and packaged into λ heads in vitro (*13*). The phage containing packaged chimeric DNA was used to transduce the *E. coli* K12 restrictionless strain C600NR(λcI857)*recA*, which carries a thermally inducible prophage. Ampicillin-resistant colonies were selected at 30°C, transferred into the same selective medium, and incubated at 30°C overnight to provide a master plate. The plate was then shifted to 42°C to induce the prophage and lyse the cells (*14*). The bacteria were transferred to nitrocellulose filters and tested by an immunoblot radioassay (*15*) for the presence of M6 protein. In this assay, ^{125}I-labeled staphylococcal protein A is used to locate antigen-antibody complexes. Antiserum made to purified M6 protein [that is, extracted by phage lysin (*5, 16*) and absorbed extensively with *E. coli* K12] was used to identify colonies producing the M6 molecule.

Among 335 colonies screened, one reacted strongly with antiserum to purified M6 protein. The chimeric plasmid present in this strain was named pJRS42. The ability of this *E. coli* clone to produce this protein was stably maintained on subculture in ampicillin-containing medium.

To identify the molecule in the *E. coli* clone that reacts with antiserum to M6, an immunoblot procedure (*15*) was used.



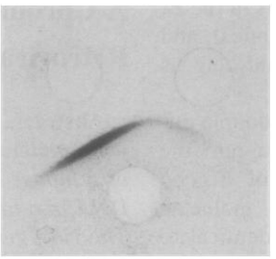


Fig. 1 (left). Immunoblot analysis of cloned and lysin-extracted M6 protein. The proteins in whole cell lysates of *E. coli* C600NR(pJB8) and C600NR(pJRS42) and purified lysin-extracted M6 protein (LysM6) were separated on a 12 percent SDS-polyacrylamide gel (*21*). Molecular weight standards were run in a parallel well. The section of the gel containing the standards was stained with Coomassie brilliant blue R, while the remainder was electrophoretically transferred onto nitrocellulose. The proteins bound to the membrane were reacted with antiserum to lysin-extracted M6 protein. Bound antibody was detected with alkaline phosphatase–conjugated goat antiserum to rabbit immunoglobulin G (Sigma). The bands were visualized (*22*) with indoxyl phosphate as the alkaline phosphatase substrate and nitro blue tetrazolium as the chromophore. Subsequent experiments with monoclonal antibodies to the lysin-extracted M6 molecule revealed a pattern of reactivity identical to that observed with the polyclonal antiserum. The molecular weight standards were phosphorylase b (*94K*), bovine serum albumin (*67K*), ovalbumin (*43K*), carbonic anhydrase (*30K*), and soybean trypsin inhibitor (*20K*). Fig. 2 (above). Immunodiffusion comparison of M protein of *E. coli* with that extracted by lysin from streptococcus. The lower well contained unabsorbed rabbit antiserum prepared against lysin-extracted M6 protein synthesized by the streptococcus. The upper left well contained purified lysin-extracted M6 protein and the upper right well contained M6 protein from the *E. coli* strain C600NR(pJRS42), which was partially purified by chromatography on DEAE- and carboxymethyl cellulose (*23*). The reaction was performed in a 1 percent agar gel prepared in 50 m*M* barbital buffer (*p*H 8.6). The gel was dried and stained with Coomassie blue before being photographed.

Strains C600NR(pJB8) and C600NR (pJRS42) were placed in Todd-Hewitt broth containing ampicillin, grown at 30°C to late log phase, washed twice, lysed with EDTA-lysozyme, frozen in dry ice and ethanol, and thawed quickly at 37°C. After treatment with deoxyribonuclease, the cellular debris was removed by centrifugation at 10,000*g* for 30 minutes, and the extract was passed through a 0.45-μm Millipore filter and dialyzed against 50 m*M* ammonium bicarbonate. Equivalent protein concentrations, as determined by the Folin reaction (*17*), were applied to a 12 percent polyacrylamide gel containing sodium dodecyl sulfate (SDS). A standard preparation of purified M6 protein extracted from the type 6 streptococcus by solubilization of the cell wall with phage lysin (*5, 12*) was applied to an adjacent well. After electrophoresis, the separated proteins were transferred to nitrocellulose (*15*) and analyzed with antiserum to lysin-extracted M6 protein that had been absorbed with *E. coli*.

The antiserum to M6 reacted with both the M6 control and the extract of the *E. coli* clone containing pJRS42, but not with the extract from the parent *E. coli* strain containing only the pJB8 vector (Fig. 1). The purified lysed M6 preparation showed a multiple banding pattern previously observed with the M6 molecule (*18*), which is probably due to degradation during extraction and purification (*5*). An identical pattern is also observed in a gel stained with Coomassie blue (*19*). The three major bands correspond to apparent molecular weights of 51,000, 52,000, and 53,000. The size heterogeneity of the M6 preparation probably results from differences at the carboxyl-terminal region of the protein since, during amino-terminal sequence analysis of this preparation by sequential degradation, only a single amino acid residue was released at each step (*16*). Since the bands from the pJRS42-containing clone that react with antibodies to M6 are all larger (molecular weight, 55,000, 57,000, 59,000) than any from the streptococcal preparation, it seems likely that pJRS42 contains the entire structural gene for the M6 protein. This is supported by the fact that this molecular size correlates well with the reported size of M protein secreted from protoplasts and L forms of type 12 streptococci (molecular weight, 58,000) (*20*). Thus, the proteins in the *E. coli* preparation may be closer to the size of the intact native M molecule than those released by lysin extraction of the streptococcus. It is also possible that the M protein that appears on the external surface of the streptococcus is synthesized with a leader sequence that is removed during secretion. If this leader sequence is not removed from the M protein extracted from lysed *E. coli*, the apparent molecular weight of the *E. coli* protein would be larger than that of the protein isolated from the streptococcus. Other explanations can be proposed for

the presence of the three bands in the *E. coli* extract, but physical, chemical, and DNA sequence analysis should help us select the correct one.

The results of Ouchterlony double diffusion experiments (Fig. 2) support the conclusion that the extract of *E. coli* carrying pJRS42 contains a molecule with antigenic determinants identical to those of streptococcal M6 protein.

Thus the M6 protein synthesized in *E. coli* has the same type-specific determinants as the M protein extracted from type 6 streptococci, although the *E. coli* product has a higher apparent molecular weight. The expression of the M protein gene in *E. coli* will allow us to dissect the molecular structure of the M molecule, which may lead to an understanding of how M proteins function to prevent phagocytosis as well as the mechanism by which streptococcal M types change during evolution.

Note added in proof: As determined by bactericidal assay, rabbits immunized with purified M6 proteins produced by the *E. coli* develop antibodies which allowed phagocytosis of type 6 streptococci.

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X Chromosome–Linked Transmission and Expression of Retroviral Genomes Microinjected into Mouse Zygotes

Abstract. *A genomic clone consisting of the Moloney leukemia proviral genome with moderately repetitive mouse sequences was microinjected into the pronucleus of a mouse zygote. An animal was derived that carried multiple copies of proviral DNA in a tandem array. No evidence for homologous recombination was obtained. The viral genome was expressed in this animal and was transmitted as a single unit to its offspring. Subsequent breeding studies revealed that the proviral DNA had integrated on an X chromosome.*

The introduction of viral (*1–7*) and cellular (*8–14*) genes into mouse embryos has been used as a means to study gene regulation during mammalian development. In our laboratory, the Moloney strain of leukemia virus (M-MuLV) was inserted into the germ line of mice and used as a model gene, because the activation of an integrated retroviral genome can be easily monitored in animals. A number of different mouse substrains, each carrying a single M-MuLV provirus at a different chromosomal locus, have been derived by exposing embryos to the infectious virus (*6*). In contrast, nonviral genes microinjected into the pronucleus were frequently carried as multiple copies integrated in tandem in the genomes of the resulting animals (*8–14*). We compared exposure of embryos to infectious virus with microinjection of cloned proviral DNA into the pronucleus as alternative means of introducing genes into the germ line. We also investigated the effect of repetitive sequences flanking the provirus on integration.

The DNA we used consisted of the linearized pMov-3 Eco RI fragment (Fig. 1A) lacking the pBR322 vector sequences. This genomic clone was obtained from the Mov-3 substrain of mice (*15*), in which infectious virus is regularly activated, with development of viremia and leukemia (*5*). The pMov-3 clone, which consists of the entire M-MuLV genome and 8 kilobases (kb) of moderately repetitive adjacent mouse sequences, is highly infectious when transfected to 3T3 cells (*15*).

Fertilized eggs from C57BL/6 females mated with C57BL/6 males were collected on the morning of the mating. One pronucleus was injected with cloned DNA by means of a glass micropipette. Of 150 eggs injected with approximately 1 picoliter of DNA solution at a concentration of 60 μg/ml, 92 survived the injection procedure and were transferred to the oviducts of pseudopregnant foster mothers and allowed to develop to term. Twelve individuals were born and were screened at age 6 to 8 weeks for virus expression by radioimmunoassay of the serum (*16*). One mouse (No. 69) was viremic. Partial hepatectomies were performed. The DNA was extracted, cleaved with Eco RI, and subjected to blot hybridization analysis. The injected sequences were detected by hybridization with the Hpa II–Hpa II fragment (Fig. 1A) derived from the 5′ flanking cellular sequences of the pMov-3 clone; this fragment cross-hybridizes to multiple bands of normal mouse DNA (Fig. 1B) (*15*). This probe detects the M-MuLV provirus carried in Mov-3 mice as a 16.8-kb fragment (lane b in Fig. 1B) (*15*). An intensely hybridizing fragment of the same size was detected in the DNA of female No. 69 (lane c), indicating that the injected DNA had been integrated, with the Eco RI sites of the flanking sequences preserved. The other nonviremic animals did not show these sequences. By comparing the intensity of this band to the single copy of proviral DNA at the Mov-3 locus (lane b in Fig. 1B), we estimate that mouse No. 69 contained approximately 10 to 20 integrated copies of proviral DNA [see also (*17*)]. When the filter in Fig. 1B was hybridized to a M-MuLV–specific probe, the results obtained with the flanking probe were confirmed (data not shown).

The arrangement of the integrated DNA was analyzed with Hind III digestion. Figure 1C shows a predominant 9.4-kb band, which was to be expected if most of the injected DNA molecules had integrated in a tandem head-to-tail arrangement (compare Fig. 1A). In addition, a weak 14.6-kb band was seen, indicating that some of the molecules had integrated in a head-to-head arrangement at the same integration site.

The 8-kb band corresponding to the site where M-MuLV was inserted in Mov-3 mice was present in the DNA of animal No. 69 (lane c in Fig. 1B) with the same intensity as is found in normal mouse DNA (lane a in Fig. 1B); this indicates that the injected pMov-3 sequences had not integrated at the Mov-3 locus [compare the DNA from a heterozygous Mov-3 animal (lane b in Fig. 1B), which shows half the intensity of the 8-kb fragment from mouse No. 69, and, in