jugating factors (*25*). It is possible that both Nα-acetylated proteins and proteins with free NH_2-termini that do not use this residue as a recognition marker share the same ubiquitin-conjugation factor or factors.

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27. Degradation was measured in reaction mixtures (50 μl) containing 220 μg of crude reticulocyte fraction II and 3 μg of ubiquitin (Sigma), or 30 μl of reticulocyte lysate [prepared as described (*2*) except that the ATP-depleting step was omitted] as described (*6*). Reaction mixtures without ATP and ATP-regenerating system were incubated in the presence of 0.5 μg of hexokinase (Boehringer Mannheim) and 10 m*M* 2-deoxyglucose. The identity of each protein and the state of its NH_2-terminal residue (free or blocked) were determined by automated Edman degradation. A gas-phase sequenator (Applied Biosystems, Inc. model 470A) was used for the degradation (*28*). The respective phenylthiohydantoin–amino acid derivatives (PTH-aa) were identified by reversed-phase high-performance liquid chromatography (RP-HPLC) in an on-line fashion with a PTH-aa analyzer (Applied Biosystems, Inc., model 120A) fitted with a Brownlee (PTH-C18 column (internal diameter, 2.1 mm). Samples of protein (20 μg) were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), according to the procedure of Laemmli [(*29*); the histone molecule was separated using a modification of the Laemmli system as described (*30*)]. Coomassie blue staining of samples separated independently showed that each protein was at least 90% pure. The samples were then transferred onto polyvinylidene difluoride paper (*31*) (Immobilin, Millipore), and the appropriate bands were cut and sequenced directly in an NH_2-terminal fashion. For those samples that failed to show free NH_2-termini (histone H2A, actin, and α-crystallin), solution-phase digestions were carried out with *N*-tosyl-l-phenylalanine chloromethyl ketone-treated trypsin (Worthington). RP-HPLC purified internal fragments were then sequenced in an NH_2-terminal fashion in order to substantiate the presence of the desired protein. The identity of all proteins used was confirmed.
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32. Affinity-purified E1 (0.5 mg) (*12*) was injected into a goat in Freund's complete adjuvant (primary immunization). Immunization was performed again every 4 weeks with 100 μg of E1 in Freund's incomplete adjuvant. After the third immunization, the animal was bled on a regular basis 7 days after each injection. Serum (20 ml) was affinity-purified on a column of 10 ml of Protein G–Sepharose as described for purification of IgG on Protein A–Sepharose (*33*). The IgG fraction was then subjected to affinity purification on affinity-purified rabbit E1 (*12*) immobilized to CH-activated Sepharose (Sigma; 12.5 mg of protein per 3 ml of matrix) as described (*33*).
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Adipsin and Complement Factor D Activity: An Immune-Related Defect in Obesity

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Adipsin is a serine protease that is secreted by adipocytes into the bloodstream; it is deficient in several animal models of obesity, representing a striking example of defective gene expression in this disorder. Recombinant mouse adipsin was purified and its biochemical and enzymatic properties were studied in order to elucidate the function of this protein. Activated adipsin has little or no proteolytic activity toward most substrates but has the same activity as human complement factor D, cleaving complement factor B when it is complexed with activated complement component C3. Like authentic factor D, adipsin can activate the alternative pathway of complement, resulting in red blood cell lysis. Decreased (58 to 80 percent) complement factor D activity, relative to lean controls, was observed as a common feature of several experimental models of obesity, including the *ob/ob*, *db/db*, and monosodium glutamate (MSG)–injected mouse and the *fa/fa* rat. These results suggest that adipsin and the alternative pathway of complement may play an unexpected but important role in the regulation of systemic energy balance in vivo.

THE ADIPOCYTE SERVES AS THE MAJOR repository of energy stores in higher organisms. The hormonal and biochemical control of many of the steps of lipogenesis and lipolysis in adipocytes in normal and diseased states remain to be elucidated. Molecular cloning has been used to identify gene products that may be important in adipocyte metabolism, including adipsin, a member of the serine protease gene family (*1*, *2*).

Adipsin messenger RNA (mRNA) is tissue-specific; it can be detected primarily in fat tissue (*2*) and sciatic nerve (*3*). The encoded protein has several features suggesting that it can function as an active serine protease, including a catalytically ac-

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tive center (charge relay system), an apparent activation peptide, and cysteines positioned to form necessary disulfide bonds (*2*). Adipsin is constitutively secreted from cultured adipocytes, and most of this protein is found in the serum in vivo (*3*).

A possible function for adipsin in the control of adipose tissue biology or energy balance is suggested by changes in the amount of adipsin during physiological and pathological alterations of adipose tissue function. Adipsin mRNA in fat is moderately (two- to fivefold) increased in catabolic states such as fasting and insulin-dependent diabetes (*4*). Large decreases (95 to 99 percent) of this mRNA occur in both genetic and chemically induced models of rodent obesity such as *ob/ob* (obese), *db/db* (diabetic), or MSG-injected mice (*4*). The amount of adipsin in the serum also decreases. The change in adipsin mRNA is not universal to all obesities in that little or no change was observed in a model of obesity based on pure overfeeding (cafeteria-fed rat) (*4*).

The suppression of adipsin expression in the genetically obese mice appears to be a consequence of neuroendocrine factors and specific cis-acting sequences in the adipsin gene. High concentrations of glucocorticoids in the blood of the *ob/ob* mouse contribute to this suppression (*5*), while experiments with transgenic obese mice indicate that 1000 bases of 5′ flanking sequence from the adipsin gene are sufficient to decrease expression of a reporter gene in both the *db/db* and MSG-injected mouse (*6*).

We have purified recombinant murine adipsin and find that it has an activity qualitatively identical to that of factor D of the human alternative complement pathway. Furthermore, a deficiency in circulating factor D activity appears to be a common feature of several independent models of rodent obesity.

Adipsin protein was expressed from a complementary DNA (cDNA) that was inserted into two expression vectors: baculovirus vector pAC373 (*7*, *8*) and the human metallothionein-based vector pMTpn (*9*, *10*). These vectors were expressed in Sf9 insect cells and Chinese hamster ovary (CHO) cells, respectively.

Adipsin from baculovirus-infected insect cells was purified by lectin affinity and ion exchange chromatography (Fig. 1A). The purified protein appears as two bands—32 and 34 kD—both of which bind antibodies to an adipsin peptide (*3*). Both bands appear to be glycosylated with mannose containing carbohydrate because (i) they bind to the mannose-specific lectin concanavalin A and (ii) they can be converted to the predicted size of the unglycosylated protein (26 kD) by treatment with endoglycosidase F.

Adipsin produced by stably transfected CHO cells was purified by ammonium sulfate precipitation, lectin affinity chromatography, and ion exchange high-performance liquid chromatography (HPLC)(Fig. 1B). The purified protein consists of a 37- and 44-kD doublet identical in electrophoretic mobility to authentic adipsin secreted by cultured adipocytes. Their size and binding to concanavalin A suggest that the recombinant protein is extensively glycosylated.

Adipsin mRNA encodes an activation peptide that would be present in the zymogen (precursor) form of this protein (*2*). Amino acid sequence determinations indicate that baculovirus-produced protein is purified as the zymogen, while CHO-expressed material is purified as the predicted activated protease (*11*, *12*). It is assumed that either intra- or extracellular CHO cell proteases are responsible for removal of the activation peptide.

Purified CHO-expressed adipsin has no detectable proteolytic activity toward various pure and complex protein substrates (*13*) and very low catalytic activity toward synthetic di- and tri-peptide protease substrates (*14*). This lack of broad proteolytic action suggested that adipsin could be functionally similar to human complement factor D, its closest relative in the serine protease family and a protein of extremely narrow

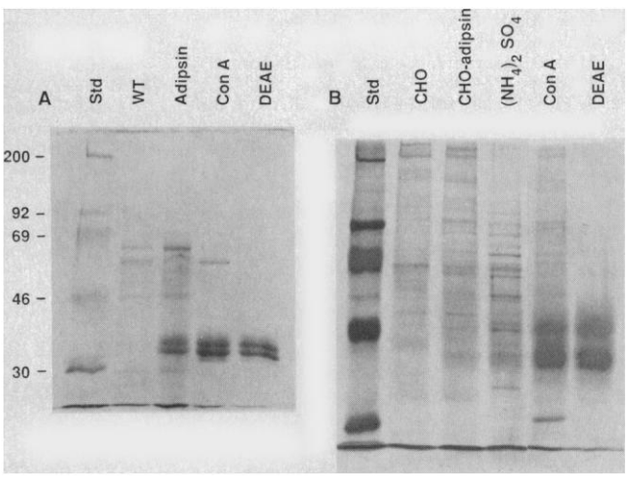

Fig. 1. Adipsin purification from baculovirus and mammalian expression systems. (**A**) Baculovirus expression. Recombinant baculovirus expressing adipsin cDNA were identified and purified (*7*), and Sf9 cells were infected at a multiplicity of 9. Secreted adipsin was purified by chromatography on concanavalin A–Sepharose and DEAE cellulose (*32*). Fractions at various stages of this purification were analyzed by 10% SDS-PAGE and stained with Coomassie blue. WT, conditioned medium from wild-type baculovirus infection; Adipsin, conditioned medium from infection with adipsin cDNA containing baculovirus vector; Con A, after concanavalin A column; DEAE, after DE-52 column. Molecular standards (STD) are shown at the left, including myosin heavy chain (200 kD), phosphorylase b (92 kD), bovine serum albumin (69 kD), ovalbumin (46 kD), and carbonic anhydrase (30 kD). (**B**) CHO cell expression. CHO cells were stably transfected with the pMTpn-adipsin vector (*9*), and expressed adipsin was purified from conditioned medium by ammonium sulfate precipitation, concanavalin A and DEAE chromatography (*33*). Proteins at various stages of the purification were analyzed by 10% SDS-PAGE and stained with silver. CHO, conditioned medium from untransfected CHO cells; CHO-adipsin, conditioned medium from CHO cells stably transfected with pMTpn-Adn; $(NH_4)_2SO_4$, the saturated ammonium sulfate pellet. The same size standards are used as in (A).

```
                          20                  40
Adipsin     ILGGQEAAAHARPYMASVQVNGTHVCGGTLLDEQWVLSAA
            |||| || |||||||||||| || | ||| |  ||||||||
COMP D      ILGGREAEAHARPYMASVQLNGAHLCGGVLVAEQWVLSAA

                          60                  80
Adipsin     HCMDGVTDDDSVQVLLGAHSLSAPEPYKRW-YDVQSVVPH
            ||      |  |||||||  |  |||       |   |||
COMP D      HCLEDA-ADGKVQVLLGATHLPQPEPXXXITIEVLRAVPH

                         100                 120
Adipsin     PGSRPDSLEDDLILFKLSQNASLGPHVRPLPLQYEDKEVE
            | | ||    || |  ||  | ||| |||||  |  |  |
COMP D      PDSQPDTIDHDLLLLQLSEKATLGPAVRPLPWQRVDRDVA

                         140                 160
Adipsin     PGTLCDVAGWGVVTHAGRRPDVLHQLRVSIMNRTTCNLRT
            |||||||||||  | |||||||  |         | ||  |
COMP D      PGTLCDVAGWGIVNHAGRRPDSLQHVLLPVLDRATCRL--

                         180                 200
Adipsin     YHDGVVTINMMCAESNRRDTCRGDSGSPLVCGDAVEGVVT
            | | |     |||||||||  | ||||  |||||   |||||
COMP D      Y-D-VLRL--MCAESNRRDSCKGDSGGPLVCGGVLEGVVT

                         220     228
Adipsin     WGSRVCGNGKKPGVYTRVSSYRMWIENI
             |||||||  ||||  ||||  |  ||
COMP D      SGSRVCGNRKKPGIYTRVATYAAWIDHV
```

Fig. 2. Amino acid sequence alignments of human complement D and mouse adipsin. The human complement D sequence is from (*34*). The mouse adipsin sequence is from (*2*). Sequence alignment was performed with the program Local (*35*). There are 140 exact matches out of 228 amino acids (61%).

substrate specificity (*15*). Whereas murine adipsin shares 30 to 40% amino acid similarity with most other serine proteases, it has 61% amino acid similarity with human factor D (Fig. 2). This sequence similarity is distributed throughout the molecule.

Factor D is a key obligatory component of the alternative pathway of complement, a complex series of reactions that can ultimately result in the lytic attack of foreign cells in the absence of antibody (*16*). (The alternative pathway differs from the classical pathway, which depends on the formation of an antibody-antigen complex to trigger activation.) The protein cleaves a single lysine-arginine bond in factor B, a 92-kD polypeptide, only when B is complexed with an activated form of complement component C3, either proteolytically activated C3b or chemically activated C3 (termed $C3_{H_2O}$). CHO-expressed adipsin exhibits an activity identical to that of purified human complement D, cleaving factor B into two products (Ba and Bb) that comigrate electrophoretically with authentic fragments Ba (30 kD) and Bb (62 kD) (Fig. 3). The adipsin-mediated cleavage, like that of factor D, is strictly C3 dependent. Sequencing of the NH_2-terminus of the Bb fragment produced by adipsin indicates that this protein cleaves the identical peptide bond as does factor D (Arg^{234}-Lys^{235}) (*17*).

The catalytic efficiency of recombinant adipsin is similar to that of factor D. In dose-response experiments with an electrophoretic assay, adipsin has about one-third of the activity of human factor D (calculated on a molar basis). As the B and C3 being used are human, not murine, this result suggests that adipsin has an optimal catalytic rate reasonably close to that of factor D. Adipsin's similarity to factor D could also be extended to a functional equivalence in complement-mediated cell lysis. In both factor D–dependent RD (*17*) and E_RC3b (*18*) assays, adipsin triggers red cell lysis (*19*).

Several experiments (*20*) indicate that the factor D–like activity of purified adipsin preparations is an intrinsic property of the adipsin molecule. Culture supernatants from CHO cells that were not transfected with the adipsin expression vector contain no factor D activity whereas the factor D activity from transfected cells can be easily detected by the cleavage of ^{125}I-labeled factor B (*20*). Polyclonal antibodies to the purified, baculovirus-expressed adipsin completely inhibit the factor D activity of CHO-expressed adipsin while other antibodies have no detectable inhibitory effect (*21*).

Adipsin in serum is reduced in animal models of both genetic (*db/db*, *ob/ob*) and acquired (MSG-injection) obesity although an exact quantitation was not possible previously because the titer of antibodies to an adipsin peptide was too low (*3*). The newly generated high titer polyclonal antisera and pure recombinant adipsin protein permit a more quantitative comparison of lean and obese mice. In normal, lean mice the serum context of adipsin is between 50 and 100 μg/ml (Fig. 4). Densitometric scanning indicates a decrease of 80 to 90% in serum adipsin in the obese animals. A similar decrease of serum adipsin in the *fa/fa* Zucker rat compared to lean controls has also been demonstrated (*22*). In addition to showing the 37- and 44-kD mouse adipsin species, these new antisera also reveal a broad band of 100 kD (Fig. 4) that was not seen earlier with antibodies to adipsin peptides (*3*). This 100-kD species is also reduced in the three mouse models of obesity and was not detected by preimmune rabbit serum, suggesting that it is immunologically related to adipsin. This 100-kD species may be adipsin bound to a covalent proteinase inhibitor such as a protease nexin (*23*).

The intrinsic factor D activity of adipsin and the reduction of immunoreactive adipsin in obesity suggest that there may be significant changes in complement D activity in the sera of obese animals. As determined at Z (lytic measure) equal to one in an E_RC3b hemolytic assay, D-like activity is down 80% in the obese (*ob/ob*) mice compared to their lean controls (Fig. 5). A similar decrease in factor D–mediated red cell lysis was also observed in whole serum from the *db/db* and chemically induced (MSG) model of obesity (Table 1) relative to appropriate lean controls. The factor D activity in the Zucker obese rat was investigated with the biochemical cleavage of ^{125}I-labeled factor B (*20*); there is a 58% decrease in the ability of serum from obese animals to cleave ^{125}I-labeled factor B into Ba and Bb fragments, compared to lean controls. Thus, several independent models of rodent obesity, both genetic and acquired, are characterized by a marked change in the activity of a complement component of the immune system.

Recombinant mouse adipsin has an enzymatic activity qualitatively equivalent to that of human complement factor D. Several lines of evidence presented above suggest that adipsin is indeed the functional mouse homolog of human complement factor D. Final proof regarding the identity of adipsin

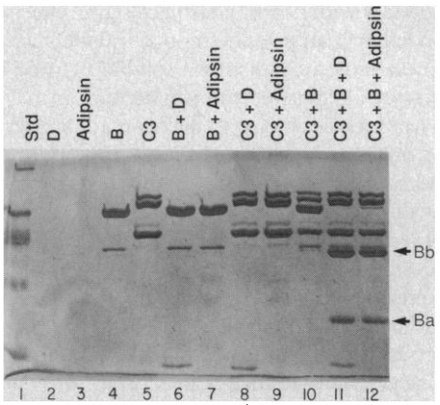

Fig. 3. Complement factor D activity in purified adipsin. Complement factors B, C3, and D were purified from pooled human plasma (*36*). $C3_{(H_2O)}$ was obtained by treating C3 with KBr (*37*). Reactions (15 μl) were held for 30 min at 37°C in 75 m*M* NaCl, 1 m*M* $MgCl_2$, 25 m*M* tris, *p*H 7.3. The following purified proteins were added: 150 ng of factor D to lanes 2, 6, 8, 11; 150 ng of adipsin to lanes 3, 7, 9, and 12; 1.5 μg of human $C3_{(H_2O)}$ to lanes 5, 8, 9, 10, 11, and 12; 1.5 μg of human factor B to lanes 4, 6, 7, 10, 11, and 12. Reactions were stopped by the addition of loading buffer and applied to a 10% SDS-polyacrylamide gel. The gel was silver stained (*38*). Adipsin and factor D were used in catalytic amounts and are barely visible. Factor D runs either with the electrophoretic front or just behind the front.

Table 1. Relative serum factor D activity in lean and obese rodents.

Model	Lean	Obese
1. *ob/ob* mouse*	1.0	0.20
2. *db/db* mouse*	1.0	0.27
3. MSG-injection*	1.0	0.29
4. Zucker rat†	1.0	0.42

*E_RC3b hemolysis assay. The standard error for the E_RC3b assays was less than 0.05. †^{125}I-labeled factor B (*20*) was cleaved with pooled sera diluted 1:4 and 1:2 from two 8-week-old obese (*fa/fa*) and lean (*Fa/Fa*) Zucker rats (*40*). Reactions were linear with respect to serum concentration and time. The standard error for the cleavage of ^{125}I-labeled factor B was less than 0.09.

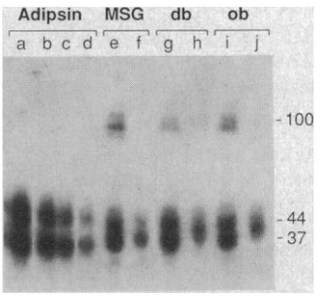

Fig. 4. Immunoblots of sera from normal and obese mice with antisera to baculovirus adipsin. Rabbit antisera to purified, baculovirus-expressed adipsin (*21*) were used at 1:500 dilution and immunoreactivity on blots was visualized by reaction with ^{125}I-labeled protein A and autoradiography. (Lanes a to d) Purified, CHO expressed adipsin containing 100, 75, 50, and 25 ng. (Lanes e to j) Serum (1 μl) from the following mice: e, lean control for MSG mouse; f, MSG-induced obese mouse; g, C57BlK/KSJ lean mouse; h, C57BlK/KSJ *db/db* obese mouse; i, C57Blk/6J lean mouse; j, C57B1K/6J *ob/ob* obese mouse. The *ob/ob*, *db/db*, and their lean littermates were 8 weeks old and were obtained from Jackson Laboratory (Bar Harbor, Maine). MSG-injected and lean controls were as previously described (*4*). The designated sera were pools of at least three animals. Obese and lean animals were age and sex matched.

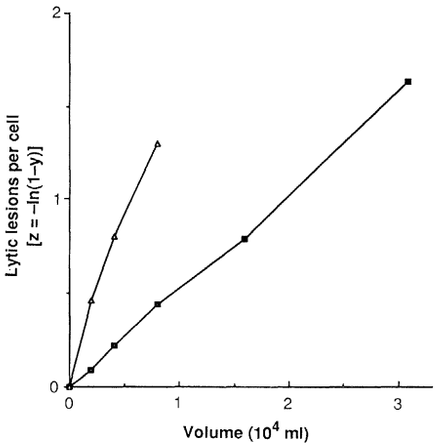

Fig. 5. Hemolytic assay for complement factor D activity in *ob/ob* and lean control mice. Serum was collected from pools of three *ob/ob* and lean control mice. Hemolytic assays for factor D activity in unfractionated serum were done with rabbit erythrocytes coated with human C3b, in the presence of excess factor B and properdin (E_RC3b assay) (*18*). Lytic sites were developed with guinea pig serum. Z, the average number of lytic lesions per cell is described in Mayer (*39*). Y is the fraction of cells lysed. -■-■-, *ob/ob* serum; -△-△-, lean control serum.

and factor D awaits the cloning, sequencing, and study of the human adipsin gene.

The observations that a fat cell product has the activity of a key complement component is highly surprising. Most complement proteins are produced by liver or cells of the immune system (*24*). Grossly increased susceptibility to infection has not been described in any of these models of obesity where we have shown decreased factor D activity, suggesting that a significant level of alternative pathway function is intact in the obese animals. As adipsin mRNA in adipose tissue decreases 30- to 100- fold in these murine models of obesity (*4*) and adipsin in the serum drops five- to tenfold, the local decrease of factor D activity in adipose tissue may be much greater than the change in activity observed in serum. Alternative sources of adipsin (*3*) that are not regulated in obesity may account for the smaller decrease in activity observed in the circulation.

Several pieces of evidence (*4*) suggest that adipsin is likely to play an active role in energy metabolism. While a connection between metabolic regulation and a protein with complement activity is not immediately obvious, the proximal, prelytic reactions of the alternative pathway of complement result in the production of several important biological mediators including C3a, C5a, Ba, Bb, and C3b (*25*). Factor D is the initial, obligatory, and rate-limiting enzyme of this pathway.

The best characterized mediators generated by the alternative complement pathway are C3a and C5a, the anaphylatoxins, small peptides with potent pharmacological effects on inflammatory and smooth muscle cells. These molecules or other soluble by-products of alternative pathway activation may have direct or indirect effects on adipocytes or other components of the energy balance systems. Furthermore, although complement pore complexes lyse bacteria and red blood cells, nucleated cells are much more resistant to their lytic effects (*26*). Sublytic complement attack increases membrane permeability, resulting in Ca^{2+} influx and the production of pharmacologically significant arachadonic acid metabolites (*27*).

The linkage of general systems of immunity and energy balance in our studies has been preceded by a few relevant examples. Tumor necrosis factor (TNFα, cachectin) has direct catabolic effects on adipocytes and potent pharmacological actions on some immune cells including leukocytes and T cells (*28*). Other immune cytokines, including gamma interferon, TNF-β, and interleukin-1, also have catabolic effects on adipocytes (*29*).

The secretion of factor D activity by adipose cells may explain some clinical observations that have linked adiposity with abnormalities in the alternative pathway of complement. Partial lipodystrophy is a human disease characterized by progressive loss of lipid from adipocytes in the upper body (*30*). The serum of patients with this disease contains an autoantibody, called C3 nephritic factor, which stabilizes C3 convertase and thus promotes the increased splitting of complement component C3 (*31*). This increases the level of a cofactor (C3b) required for adipsin or factor D action and results in a hyperactivation of the alternative pathway. Any effects of the alternative pathway on energy balance would be expected to increase in the dual presence of an alternative pathway activator like the nephritic factor and local factor D activity in adipose tissue. Thus, this lipodystrophy syndrome may present a vivid illustration of the loss of proper regulation over a complement-related process which normally influences adipose tissue physiology in a tightly controlled way. The detailed molecular mechanisms that may link energy metabolism and complement action remain to be determined.

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11. Both adipsin polypeptides from the baculovirus or CHO systems were isolated and subjected to peptide sequencing by automated Edman degradation (*12*). The baculovirus protein was sequenced for 10 cycles; the CHO protein for 22 cycles. The sequences obtained from the baculovirus-produced proteins begin with NH_2-Glu-Pro-Arg . . ., the predicted activation peptide (*2*). The CHO produced proteins begin with NH_2-Ile-Leu-Gly . . ., the predicted sequence for active adipsin (*2*).
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14. Reactions (200 μl) in 500 m*M* NaCl, 100 m*M* Hepes, *p*H 7.5, contained substrate at 200 μ*M* and 10 μg of adipsin. Fluorescence was measured after a 16-hour incubation at 37°C. Adipsin exhibited very low (less than 0.1 mole of substrate cleaved per mole of enzyme per hour) but significant activity against a small set of tripeptide substrates containing arginine in the P1 position, including *tert*-butyloxycarbonyl (Boc)-Leu-Lys-Arg-7-AMC (amino-methylcoumarin), carbobenzyloxy-Ala-Arg-Arg-AMC, and Boc-Gly-Arg-Arg-AMC. This activity could be completely inhibited by 10 m*M* diisopropylfluorophosphate (DFP). The baculovirus-expressed adipsin was inactive in these assays.
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21. New Zealand White, female rabbits were injected

subcutaneously with 500 μg of acetone-precipitated baculovirus-expressed adipsin in complete Freund's adjuvant. Animals then received two booster injections of 250 μg of baculovirus-expressed adipsin in incomplete Freund's adjuvant at 6-week intervals. Animals were bled 10 days after the last injection. The antisera so obtained reacted on immunoblots with purified adipsin expressed from baculovirus or mammalian expression systems and with purified human complement D. Immunoglobulin G (IgG) purified from these sera completely inhibited the factor D activity in mouse sera, as assayed by the cleavage of ^{125}I-labeled human factor B, while IgG from nonimmune animals had no effect.
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33. Conditioned medium from stably transfected CHO cells was collected for 48 hours in serum-free Dulbecco's modified essential medium containing .05 m*M* zinc chloride and 1 m*M* dexamethasone. Proteins that precipitated from 60% to saturation with ammonium sulfate were dialyzed against 100 m*M* NaCl, 50 m*M* tris, *p*H 7.5, and applied to a column of concanavalin A–Sepharose 4B equilibrated with the same buffer. Proteins were eluted with 0.5*M* α-methyl-mannoside in 100 m*M* NaCl, 50 m*M* tris, *p*H 7.5, dialyzed against 20 m*M* NaCl, 50 m*M* tris, *p*H 7.5, and applied to a Waters Protein-pak DEAE HPLC column at a flow rate of 1 ml/min. The column was eluted at 1 ml/min with a linear 40-min gradient of 0 to 35% 1*M* NaCl in the same buffer; adipsin eluted at 12 to 14% high salt solvent.
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Protection Against Streptococcal Pharyngeal Colonization with a Vaccinia: M Protein Recombinant

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Phagocytosis of group A streptococci requires type-specific antibodies directed against the variable determinants of the bacterial surface M protein molecule. As a step toward developing a broadly protective anti-streptococcal vaccine, a vaccinia virus (VV) recombinant was constructed that expresses the conserved region of the structural gene encoding the M6 molecule (VV:M6′). Mice immunized intranasally with the VV:M6′ virus showed markedly reduced pharyngeal colonization by streptococci after intranasal and oral challenge with these bacteria. M protein–specific serum immunoglobulin G was significantly elevated in vaccinated animals and absent in controls. A similar approach may prove useful for the identification of protective determinants present on other bacterial and viral pathogens.

APPROXIMATELY 25 TO 35 MILLION cases of group A streptococcal infections occur each year in the United States, the most common of which is acute streptococcal pharyngitis in school-age children. Up to 5% of pharyngitis cases that have gone untreated or have been ineffectively treated can lead to acute rheumatic fever, a disease that can ultimately result in cardiac damage. Although this is not a major problem in the United States, except for a recent increase in rheumatic fever cases (*1–3*), this streptococcal sequela is a significant problem in developing nations of the world. By one estimate, nearly 6 million school-age children in India suffer from rheumatic heart disease (*4*).

The ability of the group A streptococcus to cause infection is attributed primarily to the surface-located M protein, an α-helical coiled-coil fibrillar molecule (*5, 6*) that confers to the organism the ability to resist phagocytic attack (*7*). Resistance to streptococcal infection is ascribed to the development of type-specific antibodies directed to the antigenically variable NH_2-terminal determinants of the M molecule (*8, 9*). However, more than 80 antigenically diverse M proteins have been identified, thereby thwarting attempts to use NH_2-terminal M protein epitopes to develop an effective vaccine. An alternative approach, namely the induction of antibodies to epitopes representing the antigenically conserved, surface-exposed COOH-terminal region has proved ineffective in classic mouse virulence models, as these antibodies fail to initiate phagocytosis despite their ability to fix complement as effectively as type-specific antibodies (*9, 10*).

Mice immunized intranasally with the conserved region of the M protein coupled to cholera toxin B–subunit (CTB) showed a significant reduction in mucosal colonization compared with mice receiving CTB alone (*11*). The successful cloning of the streptococcal M protein gene into vaccinia virus (VV) and its expression in viral-infected cells (*12*) has allowed us to investigate the significance of a different antigen delivery system and the protective effects of antibodies directed to the conserved region of the M molecule. In the present study, recombinant VV containing the complete conserved region gene fragment of M6 protein (VV:M6′) (*13*) was used to immunize mice in-

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