with two characteristic spacings, one ~13 Å and the other ~17 Å, corresponding to five and seven atomic row spacings in ideal bulk Ag crystals. Using the valley of the supermodulation as the boundary, we labeled the two characteristic spacings as S (short segment) and L (long segment) (Fig. 4B). The peak of the L segment contains a double-ridge structure, whereas the peak of the S segment contains a single ridge.

Analysis of a strip of the STM image in the direction transverse to the atomic chains (Fig. 4C) shows that this sequence follows exactly that of the silver mean, except at two locations, one of which is construed as an extra L segment and the other as a missing S segment. These two locations can be considered as the positions where the silver mean sequence is broken. The average coherent length of the silver mean quasi periodicity, defined as the mean distance between the missing or extra segments, is ~100 Å.

The requirement of a sharply defined critical thickness for the formation of atomically flat metallic films and the preference for a silver mean over other types of quasi-periodic modulation remain unexplained. It is also unknown whether there is a causal relation between the existence of the critical thickness and the formation of the silver mean quasi-periodic structure. Further studies will be needed to resolve the many remaining questions surrounding this phenomenon.

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Mechanism of Suppression of Cell-Mediated Immunity by Measles Virus

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The mechanisms underlying the profound suppression of cell-mediated immunity (CMI) accompanying measles are unclear. Interleukin-12 (IL-12), derived principally from monocytes and macrophages, is critical for the generation of CMI. Measles virus (MV) infection of primary human monocytes specifically down-regulated IL-12 production. Cross-linking of CD46, a complement regulatory protein that is the cellular receptor for MV, with antibody or with the complement activation product C3b similarly inhibited monocyte IL-12 production, providing a plausible mechanism for MV-induced immunosuppression. CD46 provides a regulatory link between the complement system and cellular immune responses.

Measles virus kills 1 to 2 million children annually (*1*). Infection with MV is accompanied by marked and prolonged abnormalities of CMI, which contribute to increased susceptibility to secondary infections that account for most of the morbidity and mortality caused by the disease. In vivo, sensitization and expression of delayed-type hypersensitivity (DTH) responses are inhibited for several weeks after acute measles (*2*). In vitro, lymphoproliferative responses to MV antigen, recall antigen, and mitogen are suppressed, and natural killer (NK) cell activity is markedly decreased (*3*). There is both in vivo and in vitro evidence of a type 2 polarization in cytokine responses during and after measles: Production of IL-4 is increased and production of IL-2 and gamma interferon (IFN-γ) is decreased (*4*). Immunization with live-attenuated measles vaccine produces similar abnormalities in cellular immune responses (*5*). The mechanisms responsible for the marked suppression of CMI associated with measles have remained obscure.

IL-12 is critical to the development of CMI, being (i) a potent inducer of IFN-γ from T and NK cells, (ii) co-mitogenic for T and NK cells, (iii) required for the development of Th1 responses, (iv) necessary for DTH responses, and (v) an enhancer of NK cell cytotoxicity (*6*, *7*). Monocytes and macrophages are thought to be the principal IL-12–producing cells in vivo (*6*, *8*). As with other viruses that perturb CMI, such as human immunodeficiency virus (HIV), monocytes and macrophages are prime targets of MV in natural infection (*9*). Given the close match between the abnormalities of cellular immune function seen in measles and the known functions of IL-12, we hypothesized that MV infection might down-regulate the production of IL-12.

Human monocytes were isolated by countercurrent elutriation (*10*) from normal volunteers, infected with the Edmonston wild-type strain of MV, and stimulated with bacterial inducers of IL-12 production. Infection of primary monocytes with MV down-regulated the stimulated production of IL-12, both at the level of the highly regulated p40 subunit and at the level of the functional p70 heterodimer (Fig. 1) (*11*, *12*). The suppression of IL-12 was stimulus-independent, occurring whether lipopolysaccharide (LPS) or *Staphylococcus aureus* Cowan strain 1 (SAC) was used alone (Fig. 1A) or after preincubation with IFN-γ (Fig.

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1, A and B) (*13*). This suppression was not strain-specific, as several different wild-type MV isolates similarly down-regulated monocytic production of IL-12 (*14*).

The marked suppression of IL-12 production induced by MV occurred despite the fact that few monocytes were productively infected with MV. Analysis by indirect immunofluorescence for MV hemagglutinin (MV-H) and by infectious center assays indicated that less than 3% of the monocytes were productively infected at the time of secondary stimulation. Treatment of monocytes with ultraviolet (UV)-inactivated MV inhibited monocyte secretion of IL-12 (Fig. 1C) (*15*), demonstrating that productive infection is not necessary for IL-12 down-regulation by MV.

The secretion of proinflammatory cytokines and chemokines by MV-infected monocyte cultures was characterized. MV infection did not alter the stimulated production of tumor necrosis factor–α (TNF-α) (Fig. 2A), IL-6 (Fig. 2B), or macrophage inflammatory protein–1β (MIP-1β) (Fig. 2C) (*16*). The SAC-stimulated secretion of MIP-1α was suppressed by MV infection (Fig. 2D). IL-6 secretion was induced by MV infection alone ($P = 0.021$; mean production of 171 pg/ml) and was the only analyzed cytokine detectable in the absence of LPS or SAC stimulation. Effects on the stimulated production of IL-1β were highly variable, but the overall level of IL-1β production was minimal, as was expected from monocytes cultured in vitro for more than 24 to 48 hours (*17*). Thus, the inhibition of IL-12 production by MV has specificity and is not the result of a generalized failure of monocyte function.

Known endogenous inhibitors of IL-12 production include IL-10, transforming growth factor–β (TGF-β), IL-4, IL-13, and prostaglandin E2 (PGE2) (*18*). As all of these agents also suppress monocyte production of proinflammatory cytokines, their involvement in MV-mediated IL-12 suppression was not anticipated. The effect of MV on the stimulated production of these factors was assessed. In MV-infected monocyte cultures, the stimulated production of IL-10 was significantly decreased compared

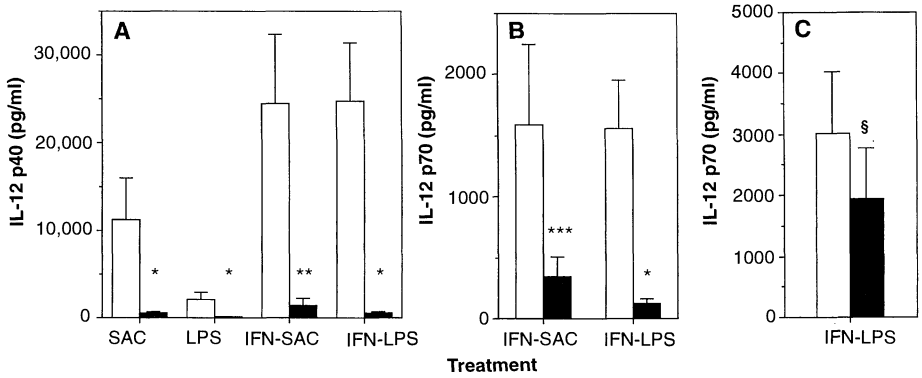

Fig. 1. MV-induced inhibition of IL-12 production by primary human monocytes. Mock-infected cultures are shown by white bars; MV-infected cultures are shown by black bars. Monocytes were stimulated with SAC, LPS, IFN-α plus SAC, or IFN-α plus LPS as indicated. (**A**) IL-12 p40 production ($n = 7$ to 11). (**B**) IL-12 p70 production ($n = 7$ to 9). (**C**) IL-12 p70 production after UV inactivation of infecting stocks ($n = 3$). Values shown are means plus standard errors. Statistical analysis of paired log-transformed data was done with the paired Student's t test. *$P < 0.0001$, **$P = 0.0001$, ***$P = 0.0009$, §$P = 0.029$, in comparison with mock infection.

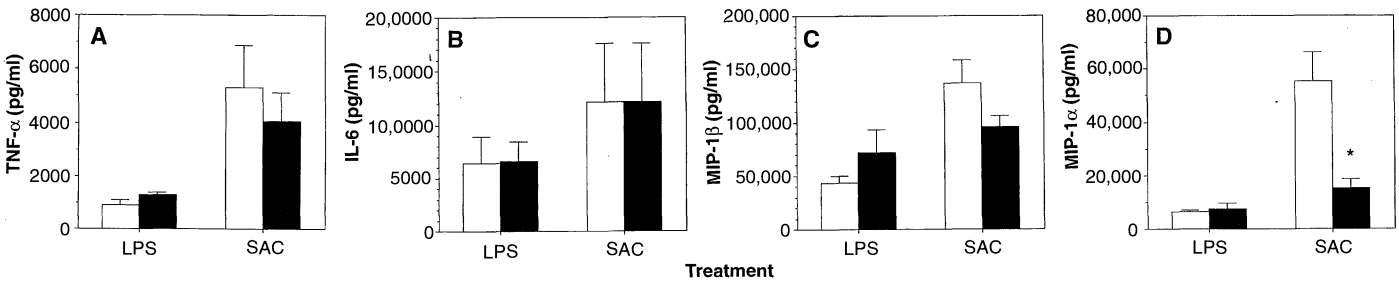

Fig. 2. Production of proinflammatory cytokines and chemokines by MV-infected monocytes in response to stimulation with LPS or SAC. (**A**) TNF-α, (**B**) IL-6, (**C**) MIP-1β, and (**D**) MIP-1α. Values shown are means plus standard errors of mock-infected (white bars) and MV-infected (black bars) monocyte cultures ($n = 3$ to 6). *$P < 0.05$.

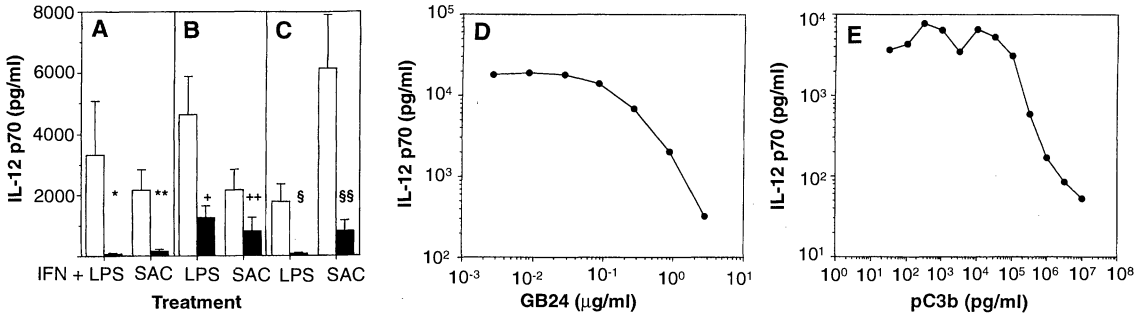

Fig. 3. Suppression of IL-12 production by ligands for CD46. (**A**) Suppression of IL-12 by GB24, a mAb to CD46. (**B**) Suppression of IL-12 by TRA-2-10, a mAb to CD46. (**C**) Suppression of IL-12 by J4-48, a mAb to CD46. Values shown are means plus standard errors of isotype-treated (white bars) and anti-CD46-treated (black bars) monocyte cultures ($n = 3$ to 5). Monocytes were stimulated with IFN-α plus LPS or SAC as indicated. *$P = 0.049$, **$P = 0.003$, +$P = 0.020$, ++$P = 0.024$, +§$P = 0.021$, and §§$P = 0.006$. (**D**) Dose response curve for GB24-mediated suppression of IL-12 production after stimulation with IFN-γ–SAC. (**E**) Dose-dependent suppression of IL-12 by dimerized C3b (pC3b). Monocytes were incubated with pC3b, prepared as previously described (*29*), before stimulation with IFN-γ–SAC. One experiment, which is representative of three, is shown.

with that of mock-infected cultures (*19*). The inclusion of neutralizing antibodies to IL-10 increased the production of IL-12 similarly in both infected and uninfected monocyte cultures (*20*), suggesting that IL-10 has no role in the specific inhibition of IL-12 effected by MV infection. Neutralization of TGF-β had no effect on IL-12 production (*21*). LPS- and SAC-stimulated PGE2 levels were not altered in infected monocytes as compared with mock-infected monocytes (*22*). As IL-4 and IL-13 are primarily products of T cells, they were not expected to play a role under these experimental conditions. Given the possibility of low-level contamination of the elutriated monocytes with T cells, however, the production of these cytokines was investigated. Neither IL-4 protein nor IL-13 mRNA was detectable by enzyme-linked immunosorbent assay (ELISA) or reverse transcriptase polymerase chain reaction (RT-PCR) assays, respectively (*23*).

Given the lack of a demonstrable role in this system for known endogenous soluble inhibitors of IL-12 and the lack of a necessary role for productive infection with MV, we hypothesized that a direct interaction between MV and its cellular receptor might be critical to MV-induced suppression of monocyte production of IL-12. The receptor for MV is CD46 (membrane cofactor protein), a cell-surface member of the regulators of complement activation (RCA) gene cluster (*24*). RCA family members control fluid phase and membrane amplification of complement activation at the pivotal C3 step and are related genetically (being tightly clustered on chromosome 1), structurally [being composed of repeating motifs known as short consensus repeats (SCRs) consisting of about 60 amino acids with four invariant cysteines and 10 to 18 other highly conserved amino acids], and functionally (binding the complement activation products C3b and C4b) (*25*). CD46 contains four SCRs. The binding site on CD46 for the MV ligand, MV-H, involves SCRs 1 and 2; C3b and C4b bind to SCRs 2, 3, and 4 (*26*).

To examine the involvement of CD46 in MV-related suppression of IL-12 production, we cross-linked CD46 on the surface of monocytes with monoclonal antibodies (mAbs). A mAb that blocks complement binding to SCRs 3 and 4 of CD46 (GB24), and mAbs to SCRs 1 and 2, which either block (TRA-2-10) or fail to block (J4-48) MV binding to CD46, were used (*27*). Cross-linking of CD46 with each of these mAbs led to a marked suppression of IL-12 production (Fig. 3, A through D) (*28*). The fact that cross-linking of CD46 directly inhibits IL-12 production suggests that cross-linking of CD46 by MV may play an important role in the suppression of CMI associated with measles through the direct down-regulation of monocyte-derived IL-12. The monokine profiles of stimulated anti-CD46–treated monocytes were identical to those of stimulated MV-infected monocytes: marked suppression of IL-12, some down-regulation of IL-10, and unaltered TNF-α and IL-6 production.

To see if endogenous complement ligands for CD46 also led to modulation of immunoregulatory monokine production, monocytes were incubated with dimerized C3b (pC3b) before stimulation. Incubation with pC3b also led to a large dose-dependent decrease in IL-12 production by monocytes (Fig. 3E), which suggests that complement activation products can directly regulate the production of IL-12.

A comparable linkage between the complement and acquired immune systems has previously been reported (*30*). Cross-linking of CD19 to membrane immunoglobulin (mIg) on B cells enhances B cell activation by reducing the number of mIg molecules that need to be ligated for activation to occur. CD19 is itself a member of a signal transduction complex on B cells, the ligand-binding subunit of which is CR2, a receptor for C3b degradation fragments and an RCA gene cluster member like CD46. Binding of a complement-opsonized immunogen to CR2 allows cross-linking of CD19 to mIg, thereby lowering the threshold for B cell activation. The present data provide evidence that the complement system not only augments humoral immune responses (through CR2-mediated up-regulation of B cell activation) but inhibits cellular immune responses (through CD46-mediated inhibition of monocyte production of IL-12). The paradigm that humoral immune responses evolved to deal with extracellular pathogens and CMI evolved to deal with intracellular pathogens is no doubt oversimplified (*31*). Nonetheless, in biasing the immune response toward humoral immunity and away from cellular immunity, the regulatory effects of complement opsonization (perforce of extracellular immunogens) provide a potential mechanistic underpinning for what remains a vital paradigm in immunology.

The presence of the regulatory linkage between the complement system and CMI provided by CD46 implies the possibility of its subversion by other macrophage-tropic pathogens. For example, infection with HIV is associated with profound defects in CMI (*32*). Mononuclear cells from HIV patients are markedly impaired in their ability to produce IL-12 (*33*). HIV efficiently activates complement, and virions as well as productively infected cells are coated with C3 activation fragments (*34*). Our data suggest the hypothesis that such complement opsonization of pathogens may lead to suppression of CMI through the inhibition of IL-12 production by monocytes.

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11. Elutriated human monocytes were infected with Vero cell–derived wild-type Edmonston (American Type Culture Collection) MV under nonadherent conditions [multiplicity of infection (MOI) = 5] or mock-infected with Vero cell lysates. Monocytes were then allowed to adhere to plastic and were subsequently cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco) at a density of 2×10^6 cells/ml (1 ml per data point) in 24-well plates (Costar). Isolation and culture were performed under LPS-free conditions (*12*). After 48 to 60 hours in culture, monocytes were stimulated with LPS (1 μg/ml) (*Escherichia coli* serotype O127:B8; Sigma) or SAC (0.0075%) (Calbiochem), with or without 18 hours of prestimulation with IFN-γ (300 U/ml) (Pharmingen). Twenty-four hours later, cell-free culture supernatants were harvested for measurement of cytokines. IL-12 p40 and p70 levels were measured by specific radioimmunoassays (RIAs) as previously described [A. D'Andrea *et al.*, *J. Exp. Med.* **176**, 1387 (1992)]. p40 was measured with the use of mAb pair C11.79 and C8.6, and p70 was measured with mAb pair 20C2 and C8.6. Both RIAs have a sensitivity of 10 pg/ml. In the absence of stimulation, there was no detectable secretion of IL-12.
12. All cell culture reagents were LPS-free to the limits of detection of the *Limulus* amebocyte lysate assay (6 to 12.5 pg/ml) (Endogen). Bactericidal permeability-increasing protein (BPI) was used in studies using reagents (such as mAb and complement) that had measurable LPS contamination [J. Weiss, P. Elsbach, I. Olsson, H Odeberg, *J. Biol. Chem.* **253**, 2664 (1978); M. N. Marra, C. G. Wilde, J. E. Griffith, J. L. Snable, R. W. Scott, *J. Immunol.* **144**, 662 (1990); M. A. Dentener, E. J. U. von Asmuth, G. F. M. Francot, M. N. Marra, W. A. Buurman, *ibid.* **151**, 4258 (1993)]. At 5 μg/ml, the recombinant NH_2-terminal fragment of BPI ($rBPI_{21}$) (XOMA) had an LPS-neutralizing capacity of >10 ng/ml [K. Meszaros *et al.*, *J. Leukocyte Biol.* **54**, 558 (1993); C. L. Karp, unpublished data]. At experimental dilutions, all reagents contained LPS amounts considerably less than 10 ng/ml.

13. Unlike peripheral blood mononuclear cells or adherence-purified monocytes (6), the production of p70 (as opposed to p40) by elutriated human monocytes is dependent on preincubation with IFN-γ (C. L. Karp, unpublished data).
14. MV strains tested in addition to wild-type Edmonston included a Finnish wild-type isolate (courtesy of A. Salmi) [R. Vainionpaa, B. Ziola, A. A. Salmi, *Acta Pathol. Microbiol. Immunol. Scand. B* **86**, 379 (1978)], as well as JM and Chicago-1 (courtesy of P. Rota).
15. MV preparations (and uninfected Vero cell lysates) were inactivated by short-wave UV irradiation for 55 min in the cold. After irradiation, stocks were kept at 4°C for at least 3 hours and were subsequently frozen before use. Such stocks were used to treat monocytes (*11*) at a (preirradiation) MOI of 5.
16. TNF-α and IL-6 were measured by ELISA (Pharmingen); ELISA measurement of MIP-1α and MIP-1β was done as previously described [H. Schmidtmayerova *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 700 (1996)].
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19. IL-10 was measured by ELISA (Pharmingen).
20. MV- or mock-infected monocytes were stimulated with IFN-γ–LPS or IFN-γ–SAC in the presence of a neutralizing mAb to IL-10 or of an isotype control (both from Pharmingen).
21. Neutralizing rabbit polyclonal (R & D Systems) and monoclonal (Genzyme) antibodies to TGF-β were used with appropriate isotype control antibodies from the same manufacturers.
22. PGE2 measurement was by RIA as previously described [L. M. Wahl, in *Manual of Macrophage Methodology*, H. B. Herscowitz, H. T. Holden, J. A. Bellanti, A Ghaffar, Eds. (Dekker, New York, 1981), pp. 423–429].
23. IL-4 measurement was by means of an ELISA with a sensitivity of 10 to 20 pg/ml (Pharmingen). IL-13 was measured by RT-PCR at 40 cycles [C. L. Karp *et al.*, *J. Clin. Invest.* **91**, 1644 (1993)].
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Structure of the Amino-Terminal Core Domain of the HIV-1 Capsid Protein

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The three-dimensional structure of the amino-terminal core domain (residues 1 through 151) of the human immunodeficiency virus–type 1 (HIV-1) capsid protein has been solved by multidimensional heteronuclear magnetic resonance spectroscopy. The structure is unlike those of previously characterized viral coat proteins and is composed of seven α helices, two β hairpins, and an exposed partially ordered loop. The domain is shaped like an arrowhead, with the β hairpins and loop exposed at the trailing edge and the carboxyl-terminal helix projecting from the tip. The proline residue Pro^1 forms a salt bridge with a conserved, buried aspartate residue (Asp^{51}), which suggests that the amino terminus of the protein rearranges upon proteolytic maturation. The binding site for cyclophilin A, a cellular rotamase that is packaged into the HIV-1 virion, is located on the exposed loop and encompasses the essential proline residue Pro^{90}. In the free monomeric domain, Pro^{90} adopts kinetically trapped cis and trans conformations, raising the possibility that cyclophilin A catalyzes interconversion of the *cis*- and *trans*-Pro^{90} loop structures.

The most distinctive architectural feature of HIV-1 and other lentiviruses is an electron-dense conical capsid core that surrounds the RNA genome at the center of the virus (*1*). The core structure is not present in freshly budded, immature virions because the membrane-bound Gag polyprotein dictates the initial steps in viral assembly and budding. Concomitant with budding, however, Gag is proteolytically processed by the viral protease to produce three new structural proteins: p17 matrix (MA), p24 capsid (CA), and p7 nucleocapsid (NC) (*2*). The processed proteins subsequently undergo a dramatic structural rearrangement, termed "maturation," in which the capsid protein condenses to form the conical core structure surrounding the NC-RNA copolymer, while the matrix protein remains bound to the viral membrane.

In addition to forming the core of the mature virion, extensive genetic analyses have revealed that HIV-1 CA performs essential roles during viral assembly and disassembly (*3–5*). Mutations and deletions within the final 80 amino acids of the 231–amino acid capsid sequence can impair or abolish viral assembly, which suggests that the COOH-terminus of CA encompasses a Gag oligomerization domain. In contrast, mutations in the NH_2-terminal two-thirds of CA often give rise to viruses that can assemble and bud but are nevertheless noninfectious. Many of these mutant viruses exhibit aberrant capsid morphologies. These observations indicate that sequences in the NH_2-terminal two-thirds of CA are essential for establishing the conical capsid core morphology and probably also play a role in viral penetration or uncoating, or both.

Another essential function of HIV-1 CA is to bind to the human cellular proline rotamase cyclophilin A (CypA). This direct interaction results in the packaging of ~200 copies of CypA into each HIV-1 virion (*6–8*). CypA packaging can be blocked by treatment of cultured virus with the immunosuppressive drug cyclosporine or its analogs (*6–9*), which bind tightly in the active site of CypA (*10*). CypA packaging can also be blocked by mutations throughout the NH_2-terminal two-thirds of CA (*6–8*). Although the precise function of CypA is not yet clear, virions lacking the enzyme appear normal by standard biochemical assays but are poorly infectious. Cyclophilin A therefore performs an essential role early in the viral life cycle (*8*, *11*), possibly accelerating the isomerization of a key capsid proline residue and facilitating viral uncoating.

High-resolution structural information

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