voke a putative GvHD mechanism *per se* but would simply act as the trigger. Indeed the heterogeneous clinical syndromes of GvHD and scleroderma cannot be superimposed, and other autoimmune disease-like syndromes are also observed in GvHD. The same principle could also apply to other ADs seen more commonly in women of child bearing age, such as rheumatoid arthritis and multiple sclerosis. One might predict that we will soon see reports of persisting microchimerism in these and other ADs.

It should also be appreciated that transplantation medicine is currently moving towards the use of microchimerism as a positive tool to induce tolerance by non-myeloablative conditioning and allogeneic hematopoietic stem cell transplantation. This approach attempts to achieve a balance between stable engraftment with reduced recipient immune function, and limited GvHD with reduced donor T-cell numbers[9]. These concepts, successful in certain animal models, are currently being investigated in the treatment of malignant and non-malignant disease, including AD. Their place relative to standard BMT remains to be established[10]. It is also hoped and expected that such a stable mixed chimeric state would allow permanent solid organ engraftment without dependency on long-term immunosuppressive agents. But clearly, we still fail to understand why some patients with full chimerism do or do not develop GvHD and why other patients with split chimerism may or may not become tolerant to the graft.

The findings of the Nelson and Artlett groups show that the 'absolute' cellular barrier of the placenta is only relative. In the thymus, some thymocytes are capable of escaping thymic control; similarly, the placenta may be leaky enabling a few immunologically reactive fetal cells to cross into the maternal circulation. The capability of such cells to initiate and sustain an autoaggressive process is dependent on class II MHC antigens, cellular, cytokine and growth factor control networks, and other hitherto unknown factors rather than on an 'all or nothing' response. Perhaps, as with low affinity autoreactive T cells that escape thymic deletion, fetal chimeric cells may become activated under certain exogenous conditions, such as concurrent infection, and may then mount an autoaggressive response. This remains speculation.

These reports are a challenge to our understanding of immunological networks. One day we should be able to use microchimerism as a tool for inducing tolerance or for diagnosing it in the quest for selective targeted treatment of AD.

1. Nelson, J.L. *et al.* Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* **351**, 559–562 (1998).
2. Artlett, C.M., Smith, J.B. & Jimenez, S.A. Identification of fetal cells in the active lesions of skin biopsies of women with systemic sclerosis. *Arthritis Rheum.* **40**, S218 (1997).
3. Gratwohl, A. *et al.* Sjogren-type syndrome after allogeneic bone marrow transplantation. *Ann. Int. Med.* **87**, 703–706 (1977)
4. Lawley, T. *et al.* Scleroderma, Sjogren-type syndrome and chronic graft-versus-host disease. *Ann. Int. Med.* **87**, 707–709 (1977).
5. Artlett, C.M., Welsh, K.I., Black, C.M. & Jimenez, S.A. Fetal-maternal HLA compatibilty confers susceptibility to systemic sclerosis. *Immunogenetics* **47**, 17–22 (1998).
6. Orlin, J.B. & Ellis, M.H. Transfusion associated graft-versus-host disease. *Curr. Opin. Hematol.* **4**, 442–448 (1997)
7. Welsh, K.I. Scleroderma: chimerism, the blind man and the scientist. *Lancet* **351**, 540–541 (1998).
8. Alamas, S.P., Wigley, F.M. & White, B. Is microengraftment with maternal or fetal cells a risk factor for the development of systemic sclerosis? *Arthritis Rheum.* **40**, S199 (1997).
9. Sykes, M. *et al.* Induction of high levels of allogeneic hematopoietic reconstitution and donor specific tolerance without myelosuppressive conditioning. *Nature Med.* **3**, 783–787 (1997).
10. Tyndall, A. & Gratwohl, A. Blood and bone marrow stem cell transplants in autoimmune disease: A consensus report written on behalf of the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant* **19**, 643–645 (1997).

[1]*Department of Rheumatology and*
[2]*Division of Hematology*
University of Basel
CH-4031 Basel, Switzerland

Two strings to the bow of Ebola virus

Two different forms of Ebola glycoprotein mediate viral entry into endothelial cells and block activation of neutrophils resulting in hemorrhage and an impaired immune response.

HANS-DIETER KLENK, VIKTOR E. VOLCHKOV & HEINZ FELDMANN

MARBURG AND EBOLA virus are filoviruses that cause fulminant hemorrhagic fever in humans and nonhuman primates, killing up to 90 percent of infected individuals. Since the discovery of Marburg virus in 1967 and its better known cousin, Ebola, a few years later, these pathogens have been cause for considerable public and scientific concern. Although it is clear from the recorded history of filovirus outbreaks that they are self-limiting and that no more than one thousand cases have been documented, Ebola's infamy is well-established. With the advent of recombinant DNA technology our knowledge of the filovirus genome and its replication strategies has significantly increased. But these discoveries do not explain why filoviruses are so deadly.

Ebola virus has a single glycoprotein gene that gives rise to different expression products (see figure). The transmembrane glycoprotein (GP)—which forms spikes on the viral surface enabling the virus to attach to and invade endothelial cells—is expressed from two reading frames by transcriptional editing[1,2]. Unedited mRNA derived from the same gene encodes a non-structural smaller glycoprotein (sGP), which is secreted by infected cells. Reporting in *Science*, Yang and co-workers[3] now present evidence to suggest that the two different forms, transmembrane GP and sGP, promote disease progression by different mechanisms. They demonstrate that sGP binds to neutrophils and inhibits their activation whereas transmembrane GP attaches to endothelial cells, enabling the virus to gain entry. Their study supports the exciting notion that a single viral gene is responsible for paralysis of the host inflammatory response and for damage to the vascular system, which promote, respectively, the rapid progression of infection and the development of hemorrhages characteristic of the disease.

The investigators analysed binding of vector-expressed sGP to normal and transformed human cell lines by immunofluorescence and found that sGP did not interact with endothelial cells but did bind neutrophils through CD16b, the neutrophil-specific form of Fcγ receptor III. They provide good evidence that sGP inhibits activation of neutrophils (although the block could be overcome using very potent activators such as phorbol ester). The interac-

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The transcriptional, translational and proteolytic products of the glycoprotein gene of Ebola virus. Transmembrane glycoprotein (GP) is encoded by two overlapping frames and is produced by transcriptional editing. It consists of subunits GP_1 and GP_2 (linked by S-S disulfide bonds) and mediates binding of Ebola virus to endothelial cells (and presumably to other susceptible cells). Significant amounts of GP_1 are released from infected cells but its binding specificity is not yet known. The secreted small glycoprotein, sGP, is expressed from unedited transcripts of the glycoprotein gene. It binds to neutrophils and appears to inhibit their activation. The first 295 amino acids of sGP and transmembrane GP are identical whereas the carboxy terminal 69 amino acids of sGP are different.

tion of sGP with neutrophils was particularly surprising because it had previously been thought that sGP served as a decoy, binding to protective antibodies and interfering with the specific immune response to transmembrane GP on the viral surface.

To determine the binding specificity of transmembrane GP, Yang and colleagues incorporated the protein into an avian retrovirus (the Moloney leukemia virus) and examined which cells were invaded by the pseudotyped retroviral particles. They showed that transmembrane GP expressed on the retroviral surface enabled the Moloney viral particles to enter endothelial cells. This confirms previous observations demonstrating that transmembrane GP mediates virus entry into cells[4] and that endothelial cells support filovirus replication[5].

As clearly indicated by the Yang study, neutrophils and endothelial cells are important targets of the Ebola virus. Of course, there are other cell types that also play an important role in its pathogenesis—cultured human macrophages can be readily infected[6] and morphological studies in monkeys and guinea pigs reveal early and extensive virus replication in cells of the mononuclear phagocytic system[7,8]. These findings led to the concept that the mononuclear phagocytic system is the primary target of Ebola virus. This view is further supported by the observation that virus-induced release of cytokines, such as TNF-α, by mononuclear cells appears to be the major cause of increased endothelial leakiness[6,9]. Filovirus infection results in depletion of lymphocytes and necrosis of antigen-presenting cells further supporting the notion that filoviral disease has an immunopathological component[7,9]. It also appears that, as the virus spreads through the organism, the spectrum of target cells expands to include endothelial cells, fibroblasts, hepatocytes, and other cell types.

There is evidence that transmembrane GP plays a crucial role in determining spread of infection. GP is cleaved at the post-translational level into the disulfide-linked fragments GP_1 and GP_2 (see figure)[10]. The same type of processing occurs with many other viral glycoproteins and is usually a precondition for virus infectivity. Interestingly, the transmembrane GP of the Reston strain of Ebola, which at least in man apparently does not cause disease, is less efficiently cleaved (because it has a different cleavage site) than the transmembrane GP of highly pathogenic strains. Such differences in glycoprotein susceptibility to cleavage are responsible for variations in the pathogenicity of avian influenza viruses and paramyxoviruses. Thus, efficient cleavage of transmembrane GP may be an important determinant of the pantropism of filoviruses.

Another secreted soluble glycoprotein, cleavage fragment GP_1, is also shed in large amounts from cells infected with Ebola virus. Notably, the highly pathogenic Marburg virus—which causes comparable disease in man and monkeys—releases GP_1 in similar amounts to Ebola but does not produce sGP because its glycoprotein gene is organized differently. Furthermore, there are highly pathogenic Ebola virus variants[7] that only secrete minute amounts of sGP (ref.10). However, the apathogenic Reston subtype produces large quantities of sGP (ref.2) but less GP_1 than other pathogenic strains[10]. So, the significance of sGP and GP_1 in the pathogenesis of filovirus infections remains to be clarified.

The work of Yang and colleagues raises several important points. Because transmembrane GP has at least some specificity for endothelial cells, it has potential as an agent for targeting retroviral vectors (or other viral vectors) carrying therapeutic genes to the vascular endothelium. This could translate into treatments for cancer or cardiovascular disease. Moreover, blocking the binding of sGP to neutrophils and of transmembrane GP to endothelial cells may constitute new approaches to treating Ebola virus infection. Developing drugs that interfere with proteolytic cleavage of transmembrane GP may be another therapeutic avenue. However, the molecular mechanisms of filovirus pathogenesis must be fully elucidated before therapeutic strategies for interfering with the different steps of infection can be successfully designed and implemented.

1. Volchkov, V.E. *et al.* GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology* **214**, 421–430 (1995).
2. Sanchez, A., Trappier, S.G., Mahy, B.W.J., Peters, C.J. & Nichol, S.T. The virion glycoprotein of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc. Natl. Acad. Sci. USA* **93**, 3602–3607 (1996).
3. Yang, Z. *et al.* Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. *Science* **279**, 1034–1036 (1998).
4. Takada, A. *et al.* A system for functional analysis of Ebola virus glycoprotein. *Proc. Natl. Acad. Sci. USA* **94**, 14764–14769 (1997).
5. Schnittler, H.J., Mahner, F., Drenckhahn, D, Klenk, H.D. & Feldmann, H. Replication of Marburg virus in human endothelial cells: a possible mechanism for the development of viral hemorrhagic disease. *J. Clin. Invest.* **91**, 1301–1309 (1993).
6. Feldmann, H. *et al.* Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. *J. Virol.* **70**, 2208–2214 (1996).
7. Ryabchikova, E. *et al.* Ebola virus infection in guinea pigs: presumable role of granulomatous inflammation in pathogenesis. *Arch. Virol.* **141**, 909–921 (1996).
8. Geisbert, T.W., Jahrling, P.B., Hanes, M.A. & Zack, P.M. Association of Ebola-related Reston virus particles and antigen with tissue lesions in monkeys imported to the United States. *J. Comp. Path.* **106**, 137–152 (1992).
9. Zaki, S.R. & Goldsmith, C.S. Pathologic features of filovirus infections in humans. *Current Topics in Microbiology and Immunology* (in the press).
10. Volchkov, V.E., Volchkova, V.A., Slenczka, W., Klenk, H.D. & Feldmann, H. Release of viral glycoproteins during Ebola virus infection. *Virology* (in the press).

Institut fur Virologie
Philipps-Universitat
35037 Marburg, Germany
email: klenk@mailer.uni-marburg.de