Infection. To determine whether leukocytes from HIV-infected patients’ alloantigen-stimulated cultures inhibited HIV-1 replication, we investigated the effect of anti-HIV supernatants derived from an alloantigen-stimulated cell line on HIV-1 reverse transcript levels in T-cell blasts infected with HIV-1. Levels of long terminal repeat-gag (LTR-gag) and LTR U3/R reverse transcripts were measured by quantitative, real-time DNA polymerase chain reaction (PCR) using primers and probe sequences previously described. Both late (LTR-gag) (Figure, panel A) and early (LTR U3/R) (Figure, panel B) reverse transcripts were significantly decreased following incubation with the alloantigen-stimulated supernatant (86% and 97% inhibition, respectively), demonstrating that alloantigen-stimulated anti-HIV activity occurs prior to reverse transcription. Figure panel C shows that the alloantigen-stimulated supernatant used in these experiments had a strong inhibitory effect on HIV-1 replication (93% inhibition), measured by p24 antigen production. These data suggest that the antiviral activity mediated by alloantigen-stimulated supernatants is distinct from the antiviral activity produced by CD8 antiviral factors (CAF), since CAF does not affect the levels of early or late reverse transcripts. β-Chemokines, CAF, and other unidentified soluble factors, released either by alloantigen-stimulated cell lines or by primary alloantigenic-stimulated peripheral blood mononuclear cells (PBMC) from healthy HIV-uninfected individuals immunized in vivo with allogeneic PBMC have been reported to inhibit HIV-1 infection. To determine whether leukocytes from HIV-infected individuals have the potential to generate anti-HIV activity after primary alloantigenic stimulation, we analyzed the effect of alloantigen-stimulated cell lines derived from HIV-infected individuals, independently of CD4+ T-cell counts, and circumvents the need for an intact CD28/B7 costimulatory pathway.

To define the molecular mechanism of inhibition of viral replication, we investigated the effect of anti-HIV supernatants from an alloantigen-stimulated cell line on HIV-1 reverse transcript levels in T-cell blasts infected with HIV-1. Levels of long terminal repeat-gag (LTR-gag) and LTR U3/R reverse transcripts were measured by quantitative, real-time DNA polymerase chain reaction (PCR) using primers and probe sequences previously described. Both late (LTR-gag) (Figure, panel A) and early (LTR U3/R) (Figure, panel B) reverse transcripts were significantly decreased following incubation with the alloantigen-stimulated supernatant (86% and 97% inhibition, respectively), demonstrating that alloantigen-stimulated anti-HIV activity occurs prior to reverse transcription. Figure panel C shows that the alloantigen-stimulated supernatant used in these experiments had a strong inhibitory effect on HIV-1 replication (93% inhibition), measured by p24 antigen production. These data suggest that the antiviral activity mediated by alloantigen-stimulated supernatants is distinct from the antiviral activity produced by CD8 antiviral factors (CAF), since CAF does not affect the levels of early or late reverse transcripts. β-Chemokines, CAF, and other unidentified soluble factors, released either by alloantigen-stimulated cell lines or by primary alloantigenic-stimulated peripheral blood mononuclear cells (PBMC) from healthy HIV-uninfected individuals immunized in vivo with allogeneic PBMC have been reported to inhibit HIV-1 infection. To determine whether leukocytes from HIV-infected individuals have the potential to generate anti-HIV activity after primary alloantigenic stimulation, we analyzed the effect of supernatants obtained from alloantigen-stimulated PBMC of HIV-infected patients on HIV-1 replication. The supernatants from the patients’ alloantigen-stimulated cultures inhibited HIV-1 and HIV-1 replication in T-cell blasts to an extent similar to that by supernatants of alloantigen-stimulated PBMC from healthy individuals (Table). Furthermore, the fraction of individuals whose culture supernatants inhibited viral replication greater than 50% was similar in patient and control cultures. Alloantigen-stimulated supernatants from an HIV-infected individual and healthy control also inhibited HIV-1 replication in monocyte-derived macrophages (94% and 84% inhibition of HIV-1 replication). These results demonstrate that alloantigen-mediated anti-HIV activity acts both in infected T cells and macrophages. Patient and control alloantigen-stimulated cultures generated similar amounts of RANTES but undetectable amounts of IFN-α. Although these control cultures produced more IL-2, IFN-α, and IL-10 than the patient cultures, the differences in cytokine production were not statistically significant (p > 0.05, Student’s t-test). Furthermore, there was no correlation between the levels of these cytokines and the inhibitory effect on viral replication (r < 0.5). No significant correlation was observed between patients’ CD4 T-cell counts (range 195-787 cells/µL) and ability of the patients’ leukocytes to generate alloantigen-stimulated HIV-suppressive activity. The role of costimulatory requirements in alloantigen-mediated anti-HIV activity has not been previously addressed. Here we demonstrate that generation of primary alloantigen-stimulated anti-HIV activity is not affected by inhibition of CD28/B7 interaction using the CTLA4Ig fusion protein (Figure, panel D), under conditions in which alloantigen-specific IL-2 production (Figure, panel E) and proliferation (Figure, panel F) are significantly inhibited (p < 0.05). These findings suggest that an intact CD28/B7 costimulatory pathway is not essential for the induction of alloantigen-stimulated HIV-suppressive activity and could-
plain the generation of this activity by alloantigen-stimulated cells from HIV-infected patients, which have been reported to exhibit immune costimulatory defects.\textsuperscript{5, 9}

These findings contribute to characterization of the molecular mechanisms and costimulatory requirements for alloantigen-stimulated anti-HIV activity that might be important for the development and application of immune-based strategies against HIV.

Ligia A. Pinto, Vesna Blazevic, Gene M. Shearer
Experimental Immunology Branch
National Cancer Institute, National Institutes of Health, Bethesda, MD

Bruce K. Patterson
Laboratory of Viral Pathogenesis
Children’s Memorial Hospital
Northwestern University Medical School Chicago, IL

Matthew J. Dolan
Infectious Disease Service
Wilford Hall USAF Medical Center
Lackland AFB, TX

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To the editor:

Correction of the PNH defect by GPI protein transfer: still an open question

Sloand et al\textsuperscript{1} report that in human red blood cells (RBCs) the paroxysmal nocturnal hemoglobinuria (PNH) defect can be corrected by transfer of glycosylphosphatidylinositol (GPI)-anchored proteins to GPI-deficient cells. It has been demonstrated before by a number of authors that cell-to-cell transfer of GPI-anchored proteins can occur, and I have no doubt that a similar event also takes place between erythrocytes and microvesicles enriched in GPI-anchored proteins. However, in my opinion, it can be questioned whether the experiments performed by Sloand et al\textsuperscript{1} warrant the conclusions that were drawn.

In Figure 1B the authors show an immunoblot using a monoclonal antibody (mAb) against CD55. This antibody reacts with more than a dozen bands (see lanes 1, 2), making it surprising that the authors are able to identify CD55 among many other bands of similar intensities in lane 3 of the same figure. The antibody apparently reacts with such a large number of proteins that the immunoblot shown is by no means a proof for the presence of CD55 in the sample—a result that the authors rely on in a number of subsequent experiments in the paper. Such a broad reactivity of a monoclonal antibody would prompt me to question its specificity or check the methodology for immunoblotting. Similarly, in Figure 1C the authors show a blot with an mAb against CD59 that reacts with 2 bands of similar intensities. Although in this case the identification of CD59 may not give rise to much criticism, one should be aware that the same antibody is used throughout the paper in flow cytometry experiments, and it is not clear with what it may react in these experiments. The situation is even more worrisome in the case of the mAb against CD55, which is also used with 2 bands of similar intensities. Although in this case the identification of CD59 may not give rise to much criticism, one should be aware that the same antibody is used throughout the paper in flow cytometry experiments, and it is not clear with what it may react in these experiments. The situation is even more worrisome in the case of the mAb against CD55, which is also used in their flow cytometric analyses.

Even more importantly, the authors claim that in order for a transfer of GPI-anchored proteins to RBCs to occur, the GPI anchors must be intact. This conclusion is based solely on their observation that treatment of the material containing the GPI-anchored proteins with bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) abolished transfer. Interestingly, however, it is well known from the literature that the GPI anchors of human RBC proteins are insensitive to cleavage by PI-PLC.\textsuperscript{2, 5} A careful study of the literature would have revealed this fact. Thus, their observation that transfer of proteins to RBCs was decreased after PI-PLC treatment cannot be explained by GPI-anchor hydrolysis, and I wonder what might have caused the altered findings.

References


