COMMENTARY

Discovery of another anti-HIV protein in the search for the CD8+ cell anti-HIV Factor

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Nearly three decades ago, soon after the recognition of AIDS and its causative agent, HIV, the first immune response against the AIDS virus was identified: an unexpected anti-HIV activity of CD8+ cells that did not involve cell killing (1). This immune response differed from the classic CD8+ cell antiviral activity in which cytotoxic T lymphocytes kill virus-infected cells (2). The CD8+ cell noncytotoxic anti-HIV response (CNAR) became evident in HIV-infected individuals who were healthy without any clinical signs but had serologic evidence of HIV infection. Although, initially, these asymptomatic individuals were expected to develop AIDS, several continued to remain healthy. Later it was determined that it takes about 10 y for 50% of infected individuals to develop AIDS (3). From these, about 5–8% are asymptomatic long-term survivors (LTS) with normal CD4+ cells and low plasma viral loads (4, 5). CNAR was discovered when the peripheral blood mononuclear cells from the LTS were placed in culture and only released infectious virus when the CD8+ cells were removed. Adding back the CD8+ cells to the infected CD4+ cells inhibited virus replication without affecting the viability of the CD4+ cells (1).

This lack of cell killing was further substantiated when the CD8+ cells were separated by a filter from the HIV-infected CD4+ cells. Virus replication was inhibited, and a soluble protease-sensitive factor, now known as the CD8+ cell antiviral factor (CAF), appeared responsible (6–8). Among the most notable properties of CAF are its production solely by CD8+ cells and its blocking HIV transcription (9–11). Importantly, its production is associated with a long-term healthy clinical state as well as prevention of HIV infection (12–14). Its activity is observed in high-risk HIV-exposed uninfected individuals (15).

The structure of CAF has been very difficult to determine. It is produced at such low levels (a 1:4 dilution of CD8+ cell culture fluid only gives 50% suppression of HIV replication in culture) that its identification has not yet been achieved (8, 11). After many years, using antibodies to known human proteins, immunologic detection techniques, and purified cytokines, no human proteins linked to CD8+ cells have been found with the characteristics of CAF (11, 16).

In PNAS, the research group led by Ian de Belle reports the surprising finding that a nuclear protein, TOE1 (target of EGR1, early growth response 1), could be CAF (17). TOE1 is found primarily in nucleoli and Cajal bodies and is involved in a variety of intracellular events, including deadenylase activity and spliceosome assembly (18, 19). TOE1 was initially cloned by the de Belle laboratory and found to bind to the p53 tumor suppressor protein, thereby playing a role in cell growth inhibition (20). The antiviral activity of this nuclear protein had been suggested by earlier restriction differentiation analyses studies indicating that TOE1 RNA was expressed at higher levels in cells showing CNAR vs. those that did not (21). This finding, however, was not confirmed by subsequent DNA microarray procedures (22, 23). The de Belle research group based their conclusion on TOE1 and CAF from the effect of TOE1 on HIV transcription, its surprising secretion by CD8+ lymphocytes, and its ability to penetrate cells and block HIV replication (17).

Notably, in their report, Sperandio et al. demonstrate that TOE1 is secreted by activated CD8+ T lymphocytes in a full length and cleaved form: Both have antiviral activity (17). The anti-HIV action results from the binding of TOE1 to the TAR sequence in the HIV promotor. Thus, it competes with the HIV Tat in the transcription process (24). This binding occurs via a long lysine/arginine nuclear localization sequence (NLS) that is similar to that found in Tat. The same basic region gives TOE1 the ability to cross plasma membranes as has been described for Tat (25, 26). The small cleaved TOE1 product has a 35-amino acid region comprising the NLS that retains the anti-HIV inhibitory activity involving TAR. This shortened form has the advantage of not being taken up by endosomal-like structures and thus can be readily available for activity (17).

Table 1. Comparison of CAF and TOE1 characteristics

Characteristic	CAF	TOE1
Secreted by normal CD8+ cells	±	+
Secreted by CD8+ cells from healthy HIV-infected subjects	+	?
Secreted by normal CD4+ cells	—	+
Associated with a clinically healthy HIV-infected state	+	?
Associated with prevention of HIV infection	+	?
Can penetrate human cells	+	+
Affects cell growth	—	±
Affects HIV transcription	+	+
Interferes with Tat activity	—	+
Directly affects viral LTR	—	+
Affects intracellular transcription factors*	+	—
Best produced after cell activation	+	+
Activity associated with proteolytic cleavage†	+	+
Affects all HIV isolates tested	+	?

CAF and TOE1 notation: ±, variable results; ?, not known.
*Related to HIV transcription.
†May not be necessary.

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The serine protease granzyme B, found in CD8+ cell cytotoxic granules, appears responsible for the cleaved TOE1 products (17). Similarly, CAF seems to undergo cleavage by a serine protease, but it is not Granzyme B (27). However, with neither CAF nor TOE1 has cleavage been proven necessary for the antiviral activity. Similar to CAF, TOE1 has shown very little cell toxicity but can inhibit cell proliferation to some extent; that did not substantially influence its inhibition of HIV replication (17).

The observations on CAF provide a background for determining the relationship of TOE1 to CAF. The CAF characteristics are different from those of TOE1 (Table 1), but the discovery of the antiviral activity of this RNA-binding protein can provide a new antiviral protein with potential clinical importance. In this regard, one needs to determine if TOE1 is associated with a clinically healthy HIV-infected state and prevention of HIV infection. It is noteworthy that the secretion of TOE1 and its ability to enter cells suggest that this protein could have a natural anti-HIV effect that warrants investigation and could be developed into an anti-HIV drug. In addition, its potential value as a vehicle transporting other drugs into cells can be appreciated.

Other CD8+ cell-associated anti-HIV cytokines have been detected in the search for CAF (16, 28–34). For example, 20 y ago, Cocchi et al. reported that a combination of beta chemokines (MIP 1α, MIP 1beta, RANTES) inhibited HIV in cell culture (28). In this case, only virus isolates using the CCR5 chemokine receptor were sensitive to the cytokine combination. In addition, another cytokine, Il-16, was reported with activity against isolates that only use the CXCR4 chemokine coreceptors (35). CAF prevents the replication of all HIV isolates tested (8, 11); the effect of TOE1 on multiple virus isolates is not yet known. Also, in contrast to the block in viral transcription by CAF and TOE1, the chemokines inhibit HIV primarily at the cell surface. TOE1 prevents the interaction of Tat with the LTR TAR region. CAF activity does not appear to involve the viral LTR, Tat, or TAR but, likely, blocks intracellular transcription factors (36, 37).

Sperandio et al. demonstrate that TOE1 is secreted by activated CD8+ T lymphocytes in a full length and cleaved form: Both have antiviral activity.

Because of the broad antiviral activity of CAF and its association with a long-term healthy HIV-infected state as well as prevention of HIV infection, its identification remains a high priority. The uncovering of other proteins that could have valuable antiviral activities has been beneficial. In this regard, the recognition of TOE1 as another potential antiviral protein, although not CAF, is noteworthy, and its possible use as a vehicle for delivering therapy is valuable. Thus, although the journey to reach the goal of identifying CAF continues, the journey itself can lead to notable discoveries.

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