Adeno-associated virus (AAV) has been a dark horse among the vector systems that are commonly considered for human gene therapy. Discovered in the middle 1960's, AAV has received relatively little attention from virologists. This was due in part to the fact that it was soon established that infection with AAV is not associated with any disease, even though most humans are routinely exposed to the virus. With no compelling medical reason to study the virus, it has remained the province of a small number of parvovirologists who have been interested in its unusual molecular biology. AAV-2, the prototype strain, has a DNA genome ~ 5,000 bases long and is incapable of replication unless a helper virus, such as adenovirus or herpes virus, is also present. The most interesting aspect of the AAV life cycle is the fact that in the absence of a helper virus, it is capable of integrating into a human chromosome. Once integrated the provirus is essentially quiescent until the cell is superinfected with a helper virus, whereupon the AAV provirus is rescued. These properties (the lack of pathogenicity and the ability to integrate) led Hermonat and Muzyczka (1) to explore ways of turning AAV into a vector for human gene therapy. They and others (2) subsequently showed that AAV can indeed integrate into chromosomes at high frequency and that only the AAV 145 base pair terminal repeats were required for vector function. More recently, Berns and his colleagues have demonstrated another intriguing property of AAV, namely, the wild-type genome integrates specifically into human chromosome 19. Whether this property can be retained in the recombinant vectors remains an open question.

In spite of the fact that AAV appears to be an ideal vector, two problems have remained. The first is that no one has developed an efficient way for generating high titer recombinant AAV virus. However, wild-type AAV produces titers similar to those of adenovirus and in principle, it should be possible to generate AAV vector stocks with the same high titers seen with adenovirus vectors. Although no one has devised a trick to accomplish this yet, it is generally believed by AAV virologists that this is a technical problem that will ultimately be solved. The second problem is arguably more important. Virtually all of the work done with AAV vectors thus far has been done in established tissue culture cells and these are well known to be a poor predictor for how a vector will behave in primary cells or animals. This problem is addressed by Walsh et al. (3) in this issue of *The Journal* and the results are encouraging for those who are interested in applying AAV vectors to human gene therapy. Walsh et al. (3) chose to study the Fanconi anemia (FA) model, specifically cases in which the defect is due to complementation group C. Patients afflicted with the disease usually die from severe bone marrow failure at an early age. Cells that are derived from FA patients are characterized by an increased number of spontaneous chromosome breaks and radials, an increased sensitivity to DNA cross-linking agents such as mitomycin C, delayed transit through the G2 phase of the cell cycle, and accelerated cell death. Walsh et al. (3) demonstrate that by all of these criteria, they are able to convert established FA cells to phenotypically normal cells after infection with an AAV vector carrying the Fanconi anemia C complementation gene. They go on to demonstrate that if CD34+ enriched cells from an FA patient are infected with the AAV vector, they show a higher survival potential. Cells incubated with virus produced a fourfold higher number of colonies compared to uninfected controls, and those that were treated with mitomycin C produced an 8–10-fold increase in the number of measurable colonies. This is the first demonstration that a genetic defect might be capable of correction by targeting human bone marrow cells with an AAV vector. Furthermore, Walsh et al. (3) suggest that because the corrected cells display higher survival than untransduced cells, the corrected cells might have a selective advantage over the patient's defective hematopoietic stem cells and be capable of repopulating a patient's bone marrow with normal cells. This strategy might be applicable to other diseases such as AIDS.

The report by Walsh et al. (3) is consistent with earlier reports that primary mouse hematopoietic cells can be successfully transduced in culture with AAV vectors carrying the neomycin phosphotransferase marker (4, 5). It is also consistent with recent work by Flotte et al. (6) which demonstrated successful transduction of primary human and rabbit airway epithelial cells by an AAV vector carrying the cystic fibrosis transmembrane conductance regulator gene. Although these observations are encouraging, it is important to point out that, thus far, no one has shown that transduction in primary cells is permanent, that is, that the AAV vector has, in fact, integrated. Indeed, there is almost no direct evidence that AAV vectors will integrate into primary cells. In addition, in the case of bone marrow gene therapy, we will have to await the results of long term bone marrow reconstitution experiments before we can be certain whether the frequency of transduction is sufficiently high to be useful for gene therapy. Finally, as AAV is taken into the clinic we may uncover other difficulties that are not apparent from what we currently know about the biology of this interesting virus.

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