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Adenoviral Infections in Hematopoietic Stem Cell Transplantation

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ABSTRACT

Adenoviruses are lytic DNA viruses that are ubiquitous in human communities. In total, 51 different serotypes with varying tissue tropisms have been identified. Adenovirus infections, although frequent, are rarely fatal in immunocompetent individuals who have potent innate and adaptive immunity. But in immunosuppressed individuals, adenoviruses are a significant cause of morbidity and mortality, with limited treatment options. In particular, pediatric recipients of allogeneic hematopoietic stem cell transplantation frequently develop infections early in the posttransplantation period. Because the endogenous recovery of adenovirus-specific T cells has proven important in controlling infection, we explore the potential of adoptive T-cell immunotherapy as a therapeutic strategy. We discuss the advantages and limitations of T-cell therapy for the prophylaxis and treatment of adenovirus infection posttransplantation.

KEY WORDS

Adenovirus • Hematopoietic stem cell transplantation • Immunotherapy

ADENOVIRUS BIOLOGY

Adenoviruses are ubiquitous, nonenveloped, lytic DNA viruses. The 51 different human serotypes have been divided into 6 species (formerly called subgroups), A–F, based on their hemagglutination properties, oncogenic potential in rats, and DNA homology (Table 1). Infection occurs by receptor-mediated endocytosis, with most human adenoviruses entering their target cells through a primary binding interaction between the fiber protein on the virus capsid and the Coxsackie-adenovirus receptor (CAR). The exceptions to this rule include species B viruses, which interact with target cells through CD46 [1,2], and the species D serotypes 8, 19a, and 37, which bind to sialic acid residues rather than to CAR on target cells [3]. Primary binding is followed by a secondary interaction mediated by the penton base of the virus and specific integrins on the target cell surface. Once species C adenoviruses have been internalized, they travel to early endosomes, from where they escape to the cytosol in an active process triggered by the acidity of this compartment [4]. The adenoviral particles then migrate to the nucleus using the cellular microtubule network, where they bind to the nuclear pore complexes and translocate the viral genomes to the nucleus allowing the viral genes to be expressed [5-8]. The intracellular trafficking route is determined by the fiber knob domain and by the nature of the primary attachment receptor [4,9,10]. Using fiber-chimeric vectors, Shayakhmetov et al. [4] demonstrated that Ad5f35 particles gain access to the nucleus by remaining inside late endosomes. During this process, some or all of the virion proteins gain access to major histocompatibility complex (MHC) class I and II processing pathways, so that infected cells become sensitive to adenovirus-specific T-cell recognition even in the absence of subsequent virus gene expression. Ultimately, the replication of viral DNA coupled with the production of large quantities of adenovirus structural polypeptides sets the stage for viral assembly and eventual escape from the host cell (Figure 1).

The viral genome codes for more than 30 structural and nonstructural proteins [11]. The nonstructural proteins can be broadly divided into 2 groups, early and late gene products. Early viral

Table 1. *Adenovirus Species and Serotypes*

Species	Tissue Tropism	Serotypes
A	Gastrointestinal tract	12, 18, 31
B	Urinary tract, Lung	3, 7, 11, 14, 16, 21, 34, 35, 50
C	Respiratory tract	1, 2, 5, 6
D	Eye, gastrointestinal tract	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51
E	Respiratory tract	4
F	Gastrointestinal tract	40, 41

gene products mediate viral gene expression and DNA replication, induce cell cycle progression, block apoptosis, and antagonize various host antiviral measures, whereas late gene products promote virus assembly and escape. Adenovirus has sophisticated mechanisms for evading both the innate and adaptive host immune systems and devotes a large portion of its genome to this purpose [12,13]. The early protein E1A and the VA (virus-associated) RNAs block responses to interferons (IFNs), whereas E1B inhibits cellular apoptosis induced by the stress of the virus infection. The evasion genes encoded in the E3 region have various functions, including tethering MHC I molecules in the endoplasmic reticulum and inhibiting the tapasin transporter that processes peptides for presentation on MHC molecules. Both processes combine to prevent recognition and lysis of infected cells by cytotoxic T lymphocytes (CTLs). In addition, E3 proteins inhibit tumor necrosis factor (TNF)-α, Fas, and TRAIL-induced cell death by removing their receptors from the infected cell surface, thus rendering the cells invisible to activated CTLs and monocytes [12-16].

Both innate and adaptive immune responses are alerted by the infection process before the expression of immune evasion genes. The innate immune response represents the first line of defense against invading pathogens, which are recognized through a number of receptors in intracellular and extracellular compartments. The most-studied family of recognition receptors is the Toll-like receptor family, although other mechanisms of pathogen recognition exist. Ultimately, recognition of pathogen-associated molecular patterns triggers a series of events, including activation of NF-κB and signal transduction through the mitogen-activated protein kinases. These signals determine the type of immune response that will be produced and result in the transcription of host chemokines, including macrophage inflammatory protein (MIP)-2, IFN-γ–inducible protein 10, MIP-1α, RANTES, and cytokines such as interleukin (IL)-1β, IL-6, IFN-γ, and TNF-α. These molecules may have a direct antiviral response and also recruit and activate innate effector cells, such as natural killer cells, granulocytes, and monocytes, to sites of infection [17,18]. Subsequently, the adaptive immune response, mediated by T and B cells [19-22], is activated.

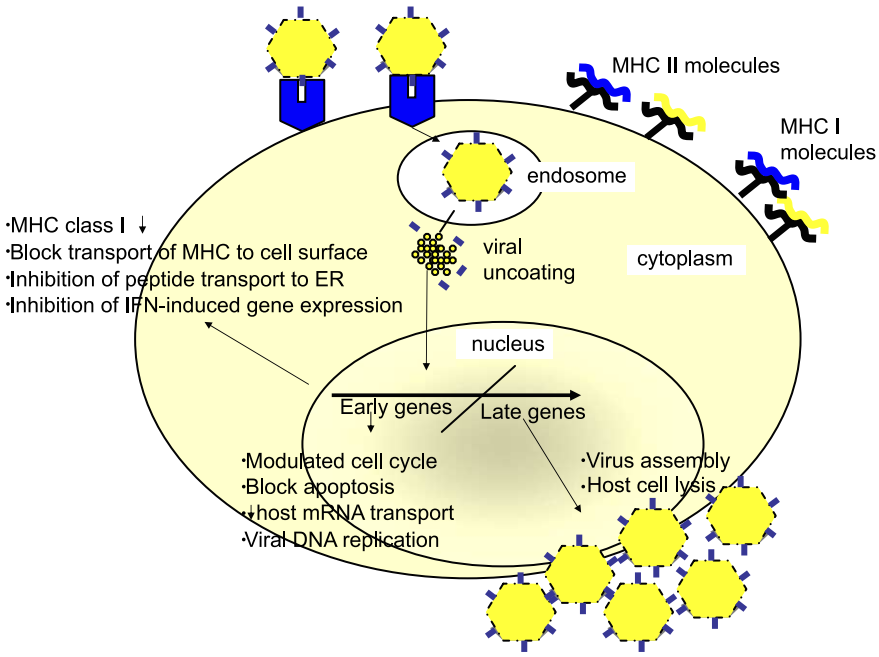

Figure 1. Adenovirus life cycle. Infection is a multistage process involving adsorption, internalization, virus uncoating and virion antigen presentation, trafficking to the nucleus, viral replication, and finally host cell lysis and virus escape. The figure indicates that virus uncoating and antigen presentation may occur before the expression of the immune evasion genes, which occurs in a temporal fashion after viral DNA entry into the nucleus. Thus the infected cell may be sensitive to lysis by virion-specific T cells before its production and the release of new infectious virions.

PATHOLOGY AND DIAGNOSIS

Many adenovirus serotypes cause morbidity and mortality in immunosuppressed individuals. The most commonly isolated serotypes are 1, 2, and 5 from species C and 34 and 35 from species B [23]. Although infection, signified by detection of viral DNA, is not always associated with disease, disease is associated with significant mortality, and antiviral therapies have limited efficacy against adenovirus.

Detection and treatment of adenovirus infections in immunocompromised patients is dependent on a sensitive means of viral identification, because adenovirus may be responsible for a wide range of clinical syndromes, including pneumonia, gastroenteritis, hepatitis, hemorrhagic cystitis, nephritis, encephalitis, and myocarditis, which are common manifestations of other post–bone marrow transplantation complications [23]. Different tissues, most commonly stool, urine, blood, and nasal washes, must be analyzed and a variety of methods have been used to diagnose adenovirus conclusively. Until recently, culturing adenovirus was the most commonly used method of detection. However, it may take up to 3 weeks for a cytopathic effect to develop, and some serotypes require special cell lines for isolation. Because early treatment with antiviral agents has a better outcome, a rapid diagnosis is required. For this reason, several groups have developed conventional, nonquantitative polymerase chain reaction (PCR) assays for the detection of adenovirus in clinical samples [24-27].

Viral quantification in clinical samples is standard practice for a number of different human viruses using real time PCR (RT-PCR) protocols [28-31]. But such a protocol is complicated for adenovirus, because of the wide range of serotypes and the consequent difficulty finding conserved sequences for primer/probe selection. Heim et al. [32] addressed this problem by designing consensus primer sequences capable of annealing to the hexon protein from all 51 different serotypes. These authors screened 218 clinical samples from multiple sites, including blood, serum, eye swabs, and feces, by conventional nonquantitative PCR and RT-PCR and found divergent results in 16 samples (15 positive only by RT-PCR and 1 positive only by conventional PCR). All of the samples that were positive by RT-PCR but negative by conventional PCR had adenovirus DNA concentrations $<10^3$ copies/run, indicating a lower sensitivity of the conventional PCR. Lion et al. [33] refined this approach by designing RT-PCR primer/probe combinations derived from both hexon and the VA RNA region, with the ability to distinguish between the 6 different adenovirus species. Identification of the infecting species may prove important, particularly if different adenovirus species are differentially sensitive to antiviral agents [34]. These RT-PCR protocols will likely become standard of care in the hematopoietic stem cell transplantation (HSCT) setting.

ADENOVIRUS INFECTIONS IN HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS

Various retrospective and prospective studies have been carried out to assess the incidence of adenovirus infection and disease in both adult and pediatric transplantation recipients and also to identify risk factors for the development of adenovirus infection and disease posttransplantation [35]. Rates of post–HSCT infection varying from 5% to 32% have been reported [33,36-41], but this broad range is likely due to different monitoring assays with differing sensitivities used by the various transplantation centers. Thus there is a need for standardization in adenovirus detection methods to definitively quantitate the incidence of adenovirus infections posttransplantation. The incidence of disease in patients with detectable adenovirus in peripheral blood can be as high as 73% [33].

Numerous groups have also attempted to identify factors that are predictive of adenovirus infection and/or disease development [35]. Lion et al. [33] found that repeated detection of adenovirus in peripheral blood using RT-PCR and rising viral load provided the most reliable means of diagnosing infection and predicting disease. Runde et al. [41] suggested that adenovirus antibodies in the donor, indicating a recent exposure, may also be a significant risk factor in the development of infection. This finding suggests that adenovirus infections post-HSCT are not always reactivations or new infections, but also may be transmitted from the donor; however, this has yet to be formally demonstrated.

The incidence of adenovirus infection is highest in children undergoing allogeneic transplantation and lowest in adults and in children undergoing autologous transplantation [33,36-40,42]. This difference may be linked to the repertoire of the immune response to adenovirus in adults and children. Adenovirus is first encountered during childhood, during which infections are frequently associated with species C viruses, so that the immune response in the early years may be quite species-specific. However, over time, the breadth of exposure to different species increases, so that immunity broadens, and, in adults, species cross-reactive CTLs that can recognize all species of adenovirus circulate in the periphery more frequently than serotype-specific CTLs [19,20,44,45]. Thus the higher incidence of infection early in the posttransplantation period in children may reflect a lack of species cross-reactive T cells. If this were the case, then risk would be expected to relate to the donor's previous exposure; however, the question of whether children receiving sibling stem cells are at greater risk of

developing an adenovirus infection than those receiving adult stem cells has yet to be addressed. A major difference between pediatric and adult transplantation recipients relates to the median time of detection (>90 days posttransplantation in adults, compared with <30 days posttransplantation in children) [36,39,42].

Once an adenovirus infection has been identified, treatment options are limited. The antiviral agents ribavirin and cidofovir can be administered in combination with tapering immunosuppression [37,40,43]. However, antivirals have controversial efficacy in vivo (as reviewed in the next section), and in some circumstances it is not feasible to taper immunosuppression because of the risk of graft-versus-host disease. Because resolution of adenovirus infections and disease has been shown to coincide with the recovery of endogenous T-cell function [46,47], adoptive immunotherapy with adenovirus-specific T cells that have been activated and expanded in vitro remains a viable (although as-yet untested) possibility. In the absence of therapeutic alternatives, various groups are currently working on developing such protocols, as discussed in more detail in the Immunotherapy section.

PROPHYLAXIS/THERAPY WITH ANTIVIRAL AGENTS

Adenovirus infections are common after allogeneic HSCT, and most patients are able to eliminate the virus without treatment, as recently demonstrated in a retrospective study by Walls et al. [48]. These authors analyzed 273 samples from 26 pediatric patients by PCR and found that 7 of 11 children with blood samples that were positive for adenovirus by PCR cleared the virus without antiviral therapy. However, it is difficult to predict which patients will spontaneously clear an infection and which patients will succumb to adenovirus disease. Treatment options are limited for the latter group. The choice of antiviral agents is restricted to ribavirin and cidofovir, but to date no study has conclusively demonstrated the efficacy of these agents as prophylaxis/treatment for adenovirus infection and/or disease [49-54].

Ribavirin, a guanosine analog with broad antiviral activity, has been used as a treatment for adenovirus infections and disease with variable results. Recently, Lankaster et al. [54] examined 4 pediatric patients who developed adenoviremia post-allogeneic HSCT and who received ribavirin at the first sign of dissemination. After antiviral treatment, viral load was measured using real-time PCR analysis. In all 4 cases ribavirin was not associated with a decrease in viral load, and in fact an increase was noted in 3 of the 4 patients [55]. Thus it would seem that ribavirin lacks significant antiviral activity in vivo. On the basis of this observation, treatment was switched from ribavirin to cidofovir in 2 of the patients, resulting in stabilization of an already extremely high viral load. Both patients eventually succumbed to disease, however.

Cidofovir is a nucleotide analog with potent in vitro reactivity against several DNA viruses. Its successful use in treating adenovirus infection and/or disease posttransplantation was reported by Bordigoni et al. [49]. Leruez-Ville et al. [56] evaluated clinical symptoms and virus load in response to cidofovir in 8 immunosuppressed patients with invasive adenovirus disease and found that 5 patients had clinical improvement and a concurrent reduction in viral load with good outcomes, but the remaining 3 did not respond and died, 2 due to adenovirus disease and 1 due to multiple infections and graft rejection. It should be noted that cidofovir treatment was started later for the 3 patients who died (a median of 18 days after the development of symptoms, compared with 8 days in those who responded) [56]. Thus it appears that cidofovir may be more efficacious in vivo than ribavirin in treating established adenovirus disease. But the time interval between the onset of symptoms and administration of treatment may be crucial, and the nephrotoxicity associated with intravenous cidofovir treatment remains a major concern. A prospective randomized, controlled trial is needed to confirm efficacy in vivo, because rapid spontaneous clearance can coincide with immune recovery post-HSCT.

CELLULAR IMMUNE RESPONSES TO ADENOVIRUS

A number of studies have linked the incidence of adenovirus disease in HSCT recipients with the lack of recovery of antigen-specific T cells [37,43,46,47,57,58]; however, to date there are no reports of immunotherapy trials for the prophylaxis and treatment of adenovirus infections in immunocompromised patients. Nonetheless, as proof of principle, one group has reported the efficacy of donor leukocyte infusion for the treatment of disease [59]. Accordingly, a number of groups have begun to analyze the T-cell immune response to adenovirus, with a view to identifying rational targets for future clinical trials. Early studies of human immunity to adenovirus, carried out by Flomenberg et al. [22,60], showed that the adenovirus-specific cellular immune response is mainly CD4+ mediated and appears to be cross-reactive among different species. An adenovirus-reactive CD8+ T-cell component was also detected in later studies [14]. Subsequently, Smith et al. demonstrated CD4+ and CD8+ adenovirus-specific T cell responses to dendritic cells infected with either wild-type Ad5 or Ad5 dl312, an E1A-deleted mutant, which expresses few if any viral genes [61], implying that at least one of the input virion proteins was stimulating memory T cells. Regn et al. [62] developed a CD40–ligand co-culture system

to infect Epstein-Barr virus (EBV)-transformed B-cell lines (LCLs) with adenovirus, then stimulated peripheral blood mononuclear cells from EBV- and adenovirus-seropositive donors weekly with autologous adenovirus-positive LCLs. Using this method, they were successful in generating CTL lines specific for both viruses, and the recognition was mediated by both CD4+ and CD8+ T cells. Using dendritic cells transduced with recombinant adenoviral vectors encoding either cytomegalovirus (CMV) pp65 or EBV EBNA3C, Hamel et al. [63] also generated polyclonal CTL lines that were bi-virus reactive. However, the response against the adenovirus component was less than that against pp65 or EBNA3C, suggesting either that adenovirus is not as immunogenic as EBV and CMV, or that the exogenous antigen-processing pathway used by adenovirus was less potent than the endogenous pathway used by the transgenes. Importantly, adenovirus-specific polyclonal T cells, activated by different methodologies, were able to recognize and kill target cells infected with viruses from multiple species [14,19,44,45,61].

More recently, various groups have begun mapping T-cell target antigens in adenovirus. When investigating whether the virion proteins hexon, penton, and fiber were responsible for stimulating adenovirus-specific T cells in vitro, Hamel et al. [63] found that hexon was strongly immunogenic in most donors, whereas penton and fiber were found to be less immunogenic. Hexon is the largest and most abundant of the structural proteins in the icosahedral adenoviral capsid. The other two major capsid proteins, penton base and fiber, form the penton complex at each virion vertex. Thus it seems that the T-cell immune response is polarized to these abundant structural antigens, which are readily available for processing and presentation to circulating T cells before the expression of immune evasion genes [64]. The fine mapping of T-cell peptide epitopes in adenovirus is also being done by various groups, and a hexon-specific CD4+ T-cell epitope has been described [21,65], whereas our group has identified multiple hexon-specific CD8+ T-cell epitopes [44]. These studies have provided potential target antigens for immunotherapy, as well as peptide and tetramer/multimer reagents for immunologic evaluation of T-cell infusions.

ADENOVIRUS CROSS-REACTIVITY

Antibody responses to adenovirus can be broadly divided into 2 types: species-specific reactivity and type-specific reactivity. Generally, species-specific antibodies are nonneutralizing, whereas type-specific responses are directed against hypervariable regions on the virus capsid and can effectively neutralize extracellular virus, thus preventing virus spread [66-69]. The main targets of type-specific neutralizing antibodies are the adenovirus structural proteins, hexon and fiber [70,71].

T-cell reactivity in humans has also proven to be cross-reactive, although most of this analysis has been carried out in bulk CTL lines [21,45,60,61]. More recently, these studies have been refined to a clonal level; Heemskerk et al. [19] analyzed CD4+ clones from cross-reactive polyclonal CTL lines activated using inactivated Ad5 (species C) as an antigenic stimulus. Of 11 T-cell clones tested, 2 reacted only with other species C viruses, 4 reacted with species B and C viruses, and 5 reacted with species A, B, and C viruses in proliferation assays [19]. It should be noted that viruses from species D, E, and F were not screened, and the antigen and epitope specificity of the clones was not identified.

Our group has also addressed the question of adenovirus cross-reactivity using a panel of CD8+ T-cell clones generated from a cross-reactive polyclonal line, reactivated with a replication-defective chimeric Ad5f35 vector [20,72,73]. The CD8+ T-cell clones were reactive against 5 hexon-specific T-cell epitopes as assessed by cytotoxicity assay. Four of these epitopes, restricted by HLA-A*1, HLA-A*2, and HLA-B*7, were processed and presented in a species B, C, D, and E cross-reactive manner, whereas the remaining epitope, recognized in the context of HLA-A*24, was specific for viruses within species C and D. Viruses from species A or F were not tested, but the epitope sequences were conserved within these species [44].

The hexon protein can be divided into two parts: the loops on the outer surface of the molecule, which are hypervariable, and the base of the protein, which is highly conserved because of packing constraints within the capsid [11,68]. Examination of the location of our hexon-specific T-cell epitopes on the hexon molecule revealed that all were located in the conserved region of hexon, irrespective of whether reactive T cells recognized viruses from 2 species or 4 species. Thus, we hypothesize that the adenovirus-specific memory T-cell pool reflects the pattern of previous exposure [74,75]. We predict that after the first exposure, T cells will be specific for both type-specific and cross-reactive epitopes; with subsequent infections, cross-reactive T cells will be expanded preferentially, so that in adults who have experienced multiple infections with various serotypes, cross-reactive epitopes will dominate. Because the pool of adenovirus serotypes is stably maintained in human populations and there is a temporal infection pattern, with most common exposure to species C viruses in childhood followed by other serotypes from the other species, species cross-reactive immunity can be developed and maintained [70,71]. This may have implications for HSCT recipients, because immunity transferred from younger donors may be less broadly cross-reactive and less pro-

tective than that from older donors. However, this has yet to be addressed in clinical studies and in studies of adenovirus-specific immunity in children.

IMMUNOTHERAPY

Adoptive immunotherapy has already proven successful as prophylaxis and treatment for EBV- and CMV-related infections and disease in HSCT recipients [76-81]. In vitro expanded CTLs have a number of advantages over standard therapy, including their lack of toxicity and ability to persist and protect during the entire posttransplantation period [77,79], but they present both scientific and practical complexities. First, the T-cell line must be generated under good manufacturing practices in a specialized cell culture facility, which is available in few hospitals. Therefore, the global usefulness of immunotherapy as an approach for the prophylaxis/treatment of viral infections is limited, although stem cell processing facilities may be adapted for cell culture. Any CTL expansion protocol must use approved reagents and supplies, and although clinical grade products are not required for phase I/II protocols, they must meet certain manufacturing requirements and be approved by federal regulatory agencies. Finally, the rules that govern the generation, safety testing, and infusion of such lines are extensive to the point of being prohibitive, and the associated expenses are considerable.

From a scientific standpoint, initiating adoptive immunotherapy protocols for adenovirus is complicated, because adenovirus T-cell immunology is less well understood and it is unclear which target antigens will be protective in vivo. Experience in other systems has indicated that a successful/efficacious CTL product should contain a combination of CD4+ and CD8+ T cells to promote in vivo persistence [77,82]; therefore, a simple and reliable method of activating and expanding adenovirus-specific polyclonal CD4+ and CD8+ CTLs must be developed. Finally, to evaluate the efficacy of T-cell infusions, it must be possible to track their function and persistence over time after infusion.

Recent advances in our understanding of adenovirus immunogenicity, as well as increasing detection of adenoviremia in immunosuppressed individuals, have spurred various groups to develop adenovirus-adoptive immunotherapy protocols that can be performed under good manufacturing practices and applied to a clinical setting. Feuchtinger et al. [57] took advantage of the IFN-γ secretion assay to isolate adenovirus-specific T cells from peripheral blood or from leukapheresis products. Peripheral blood mononuclear cells ($0.1–2 \times 10^9$) were stimulated with an adenovirus lysate for 16 hours, and then cytokine-secreting cells (median, 3.4×10^6) were isolated using the CliniMACS system. These cells were subsequently expanded in vitro using 100 U/mL of IL-2 and irradiated allogeneic feeders (5×10^6/mL) until there were sufficient cells for infusion purposes. It took a median of 18 days to reach 10^8 total cells, and these were tested in various functional assays to confirm the specificity of the lines and to assess any residual allogeneic activity. The purity at the end of the expansion phase was 85%, with some nonspecific reactivity remaining. Feuchtinger et al. [57] propose using this system in future immunotherapy protocols, because it is an easy and rapid method for isolating and expanding antigen-specific T cells that may be applicable to clinical-grade applications. However, higher purity may be required at the initial isolation step to prevent the carry-over of nonspecific T cells, and an alternative source of virus-free antigen may be required as a stimulus.

Our group has adopted a different approach to adenovirus-adoptive immunotherapy. We reasoned that because adenovirus structural proteins appear to be immunogenic in vitro, they may provide sufficient antigen stimulation to memory T cells. Adenoviral vectors are readily available as a purified clinical-grade product because they have been used for many years in gene therapy studies and as vaccines [83]. We have produced a clinical-grade chimeric Ad5f35-null vector that, after transduction into activated monocytes, is able to reactivate hexon-specific T cells [20,44]. For the in vitro expansion of the T cells, we used autologous Ad5f35 null-transduced LCL, which can readily be produced from each donor, providing an unlimited source of APCs (antigen-presenting cells) that can present adenovirus proteins [20]. Although LCLs also present EBV antigens, they maintain the adenovirus-specific T-cell component during the expansion process with only minor expansion of residual EBV-specific T cells that remain in the cultures after the first stimulation with adenovirus-transduced monocytes [20]. Because allogeneic HSCT recipients are at risk for both adenovirus and EBV, we reasoned that the infusion of a bi-virus–specific CTL line specific for both viruses would be a bonus. There are some limitations associated with this method, most notably the use of an LCL for the second and subsequent rounds of expansion. In this situation the generation of an LCL line, rarely a problem in healthy bone marrow donors, can take up to 6 weeks, causing a significant delay in the CTL generation process. Because adenoviral infections occur early in the posttransplantation period, CTLs may be most effective when administered at this time. Therefore, in the long term, other sources of APCs, such as B-cell blasts or methods using artificial APCs for expansion, may be investigated to speed up the CTL generation process.

A clinical protocol for the infusion of adenovirus-specific CTLs into recipients of allogeneic HSCT was

submitted to and approved by the Baylor College of Medicine Institutional Review Board, the Recombinant DNA Advisory Committee, and the US FDA. Patients will be enrolled on this study in the coming months, and we anticipate the patient cohort to be dominated by pediatric patients undergoing allogeneic transplantation at Texas Children's Hospital. Patients will receive CTLs regardless of adenovirus status on enrollment. Although there may be too few patients in this study to determine antiviral efficacy, we will be able to evaluate the patients' ability to reconstitute immune responses to adenovirus using immunologic assays. For this reason, we will preferentially enroll patients with an informative HLA type, that is, for which we have identified epitopes. This will enable the use of multimer and ELISPOT assays to track infused T cells in preinfusion and postinfusion blood samples and evaluate their ability to expand, reconstitute immunity to adenovirus, and persist in vivo.

Along with immunologic monitoring, extensive virologic monitoring of patients for the presence of EBV and adenovirus preinfusion and postinfusion will be performed using RT-PCR analysis of peripheral blood for EBV and blood, urine, and stool samples for adenovirus. Initially, samples will be assessed for adenovirus positivity using a generic primer–probe combination that detects all adenovirus serotypes. If an infection is detected, then the infecting species will be identified using the primer–probe combination specific to each species. This virologic monitoring will be carried out pretreatment, then weekly for 60 days postinfusion, and then in accordance with the standard of care for each patient.

PERSPECTIVES

In summary, adenovirus infections remain a major cause of morbidity and mortality, particularly in HSCT recipients. Although significant advances have been made in our ability to detect infection and even to identify the infecting species, antiviral therapies are lacking. A number of recent publications have demonstrated a link between the recovery of endogenous adenovirus-specific T cells and protection against infection and disease in vivo. Consequently, protocols for the infusion of in vitro expanded adenovirus-specific T cells as prophylaxis and/or treatment for infection and disease are being developed by a number of translational groups worldwide. This work is being facilitated by the identification of immunogenic adenovirus antigens, which will provide rational targets for in vivo studies. In addition, the list of adenovirus-specific CD4+ and CD8+ T cell epitopes is growing, allowing for the characterization of CTL lines preinfusion and facilitating follow-up analysis postinfusion. Thus, while waiting for the identification of novel and nontoxic antiviral agents that are effective against adenoviruses, adoptive T-cell therapy for adenovirus may provide the best treatment option available. Providing that the immunogenic antigens identified are protective, we can anticipate a significant reduction in the number of patients who succumb to adenovirus infections posttransplantation, as has been the case with adoptive immunotherapy for EBV.

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REFERENCES

1. Gaggar A, Shayakhmetov DM, Lieber A. CD46 is a cellular receptor for group B adenoviruses. *Nat Med*. 2003;9:1408-1412.
2. Gaggar A, Shayakhmetov DM, Liszewski MK, Atkinson JP, Lieber A. Localization of regions in CD46 that interact with adenovirus. *J Virol*. 2005;79:7503-7513.
3. Arnberg N, Edlund K, Kidd AH, Wadell G. Adenovirus type 37 uses sialic acid as a cellular receptor. *J Virol*. 2000;74:42-48.
4. Shayakhmetov DM, Li ZY, Ternovoi V, Gaggar A, Gharwan H, Lieber A. The interaction between the fiber knob domain and the cellular attachment receptor determines the intracellular trafficking route of adenoviruses. *J Virol*. 2003;77:3712-3723.
5. Greber UF, Suomalainen M, Stidwill RP, Boucke K, Ebersold MW, Helenius A. The role of the nuclear pore complex in adenovirus DNA entry. *EMBO J*. 1997;16:5998-6007.
6. Greber UF, Webster P, Weber J, Helenius A. The role of the adenovirus protease on virus entry into cells. *EMBO J*. 1996; 15:1766-1777.
7. Leopold PL, Kreitzer G, Miyazawa N, et al. Dynein- and microtubule-mediated translocation of adenovirus serotype 5 occurs after endosomal lysis. *Hum Gene Ther*. 2000;11:151-165.
8. Suomalainen M, Nakano MY, Keller S, Boucke K, Stidwill RP, Greber UF. Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus. *J Cell Biol*. 1999;144:657-672.
9. Miyazawa N, Crystal RG, Leopold PL. Adenovirus serotype 7 retention in a late endosomal compartment prior to cytosol escape is modulated by fiber protein. *J Virol*. 2001;75:1387-1400.
10. Miyazawa N, Leopold PL, Hackett NR, et al. Fiber swap between adenovirus subgroups B and C alters intracellular trafficking of adenovirus gene transfer vectors. *J Virol*. 1999;73: 6056-6065.
11. Rux JJ, Burnett RM. Adenovirus structure. *Hum Gene Ther*. 2004;15:1167-1176.
12. Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther*. 2004;15:1157-1166.
13. Wold WS, Doronin K, Toth K, Kuppuswamy M, Lichtenstein DL, Tollefson AE. Immune responses to adenoviruses: viral evasion mechanisms and their implications for the clinic. *Curr Opin Immunol*. 1999;11:380-386.

14. Flomenberg P, Piaskowski V, Truitt RL, Casper JT. Human adenovirus-specific CD8+ T-cell responses are not inhibited by E3-19K in the presence of gamma interferon. *J Virol*. 1996; 70:6314-6322.
15. Wold WS, Gooding LR. Adenovirus region E3 proteins that prevent cytolysis by cytotoxic T cells and tumor necrosis factor. *Mol Biol Med*. 1989;6:433-452.
16. Wold WS, Gooding LR. Region E3 of adenovirus: a cassette of genes involved in host immunosurveillance and virus–cell interactions. *Virology*. 1991;184:1-8.
17. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol*. 2001;19:65-91.
18. Pasare C, Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. *Adv Exp Med Biol*. 2005;560:11-18.
19. Heemskerk B, Veltrop-Duits LA, van Vreeswijk T, et al. Extensive cross-reactivity of CD4+ adenovirus-specific T cells: implications for immunotherapy and gene therapy. *J Virol*. 2003;77:6562-6566.
20. Leen AM, Sili U, Savoldo B, et al. Fiber-modified adenoviruses generate subgroup cross-reactive, adenovirus-specific cytotoxic T lymphocytes for therapeutic applications. *Blood*. 2004;103: 1011-1019.
21. Olive M, Eisenlohr L, Flomenberg N, Hsu S, Flomenberg P. The adenovirus capsid protein hexon contains a highly conserved human CD4+ T-cell epitope. *Hum Gene Ther*. 2002;13: 1167-1178.
22. Olive M, Eisenlohr LC, Flomenberg P. Quantitative analysis of adenovirus-specific CD4+ T-cell responses from healthy adults. *Viral Immunol*. 2001;14:403-413.
23. Kojaoghlanian T, Flomenberg P, Horwitz MS. The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol*. 2003;13:155-171.
24. Allard A, Girones R, Juto P, Wadell G. Polymerase chain reaction for detection of adenoviruses in stool samples. *J Clin Microbiol*. 1990;28:2659-2667.
25. Echavarria M, Forman M, Ticehurst J, Dumler JS, Charache P. PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. *J Clin Microbiol*. 1998;36:3323-3326.
26. Pring-Akerblom P, Adrian T. Type- and group-specific polymerase chain reaction for adenovirus detection. *Res Virol*. 1994; 145:25-35.
27. Pring-Akerblom P, Adrian T, Kostler T. PCR-based detection and typing of human adenoviruses in clinical samples. *Res Virol*. 1997;148:225-231.
28. Mercier B, Burlot L, Ferec C. Simultaneous screening for HBV DNA and HCV RNA genomes in blood donations using a novel TaqMan PCR assay. *J Virol Methods*. 1999;77:1-9.
29. Morris T, Robertson B, Gallagher M. Rapid reverse transcription-PCR detection of hepatitis C virus RNA in serum by using the TaqMan fluorogenic detection system. *J Clin Microbiol*. 1996;34:2933-2936.
30. Ryncarz AJ, Goddard J, Wald A, Huang ML, Roizman B, Corey L. Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. *J Clin Microbiol*. 1999;37:1941-1947.
31. Wagner HJ, Cheng YC, Huls MH, et al. Prompt versus preemptive intervention for EBV lymphoproliferative disease. *Blood*. 2004;103:3979-3981.
32. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol*. 2003;70:228-239.
33. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood*. 2003;102:1114-1120.
34. Morfin F, Dupuis-Girod S, Mundweiler S, et al. In vitro susceptibility of adenovirus to antiviral drugs is species-dependent. *Antivir Ther*. 2005;10:225-229.
35. Leen AM, Rooney CM. Adenovirus as an emerging pathogen in immunocompromised patients. *Br J Haematol*. 2005;128:135-144.
36. Baldwin A, Kingman H, Darville M, et al. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. *Bone Marrow Transplant*. 2000;26:1333-1338.
37. Chakrabarti S, Mautner V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood*. 2002;100:1619-1627.
38. Hale GA, Heslop HE, Krance RA, et al. Adenovirus infection after pediatric bone marrow transplantation. *Bone Marrow Transplant*. 1999;23:277-282.
39. Howard DS, Phillips GL II, Reece DE, et al. Adenovirus infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis*. 1999;29:1494-1501.
40. Kampmann B, Cubitt D, Walls T, et al. Improved outcome for children with disseminated adenoviral infection following allogeneic stem cell transplantation. *Br J Haematol*. 2005;130:595-603.
41. Runde V, Ross S, Trenschel R, et al. Adenoviral infection after allogeneic stem cell transplantation (SCT): report on 130 patients from a single SCT unit involved in a prospective multicenter surveillance study. *Bone Marrow Transplant*. 2001;28:51-57.
42. Flomenberg P, Babbitt J, Drobyski WR, et al. Increasing incidence of adenovirus disease in bone marrow transplant recipients. *J Infect Dis*. 1994;169:775-781.
43. Avivi I, Chakrabarti S, Milligan DW, et al. Incidence and outcome of adenovirus disease in transplant recipients after reduced-intensity conditioning with alemtuzumab. *Biol Blood Marrow Transplant*. 2004;10:186-194.
44. Leen AM, Sili U, Vanin EF, et al. Conserved CTL epitopes on the adenovirus hexon protein expand subgroup cross-reactive and subgroup-specific CD8+ T cells. *Blood*. 2004;104:2432-2440.
45. Smith CA, Woodruff LS, Rooney C, Kitchingman GR. Extensive cross-reactivity of adenovirus-specific cytotoxic T cells. *Hum Gene Ther*. 1998;9:1419-1427.
46. Feuchtinger T, Lucke J, Hamprecht K, et al. Detection of adenovirus-specific T cells in children with adenovirus infection after allogeneic stem cell transplantation. *Br J Haematol*. 2005;128:503-509.
47. van Tol MJ, Kroes AC, Schinkel J, et al. Adenovirus infection in paediatric stem cell transplant recipients: increased risk in young children with a delayed immune recovery. *Bone Marrow Transplant*. 2005;36:39-50.
48. Walls T, Hawrami K, Ushiro-Lumb I, Shingadia D, Saha V, Shankar AG. Adenovirus infection after pediatric bone marrow transplantation: is treatment always necessary? *Clin Infect Dis*. 2005;40:1244-1249.
49. Bordigoni P, Carret AS, Venard V, Witz F, Le Faou A. Treatment of adenovirus infections in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis*. 2001; 32:1290-1297.
50. Gavin PJ, Katz BZ. Intravenous ribavirin treatment for severe

adenovirus disease in immunocompromised children. *Pediatrics*. 2002;110:e9.
51. Hoffman JA, Shah AJ, Ross LA, Kapoor N. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2001;7:388-394.
52. La Rosa AM, Champlin RE, Mirza N, et al. Adenovirus infections in adult recipients of blood and marrow transplants. *Clin Infect Dis*. 2001;32:871-876.
53. Legrand F, Berrebi D, Houhou N, et al. Early diagnosis of adenovirus infection and treatment with cidofovir after bone marrow transplantation in children. *Bone Marrow Transplant*. 2001;27:621-626.
54. Ljungman P, Ribaud P, Eyrich M, et al. Cidofovir for adenovirus infections after allogeneic hematopoietic stem cell transplantation: a survey by the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2003;31:481-486.
55. Lankester AC, Heemskerk B, Claas EC, et al. Effect of ribavirin on the plasma viral DNA load in patients with disseminating adenovirus infection. *Clin Infect Dis*. 2004;38:1521-1525.
56. Leruez-Ville M, Minard V, Lacaille F, et al. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. *Clin Infect Dis*. 2004;38:45-52.
57. Feuchtinger T, Lang P, Hamprecht K, et al. Isolation and expansion of human adenovirus-specific CD4+ and CD8+ T cells according to IFN-gamma secretion for adjuvant immunotherapy. *Exp Hematol*. 2004;32:282-289.
58. Heemskerk B, Lankester AC, van Vreeswijk T, et al. Immune reconstitution and clearance of human adenovirus viremia in pediatric stem-cell recipients. *J Infect Dis*. 2005;191:520-530.
59. Hromas R, Cornetta K, Srour E, Blanke C, Broun ER. Donor leukocyte infusion as therapy of life-threatening adenoviral infections after T-cell–depleted bone marrow transplantation. *Blood*. 1994;84:1689-1690.
60. Flomenberg P, Piaskowski V, Truitt RL, Casper JT. Characterization of human proliferative T cell responses to adenovirus. *J Infect Dis*. 1995;171:1090-1096.
61. Smith CA, Woodruff LS, Kitchingman GR, Rooney CM. Adenovirus-pulsed dendritic cells stimulate human virus-specific T-cell responses in vitro. *J Virol*. 1996;70:6733-6740.
62. Regn S, Raffegerst S, Chen X, Schendel D, Kolb HJ, Roskrow M. Ex vivo generation of cytotoxic T lymphocytes specific for one or two distinct viruses for the prophylaxis of patients receiving an allogeneic bone marrow transplant. *Bone Marrow Transplant*. 2001;27:53-64.
63. Hamel Y, Blake N, Gabrielsson S, et al. Adenovirally transduced dendritic cells induce bispecific cytotoxic T lymphocyte responses against adenovirus and cytomegalovirus pp65 or against adenovirus and Epstein-Barr virus EBNA3C protein: a novel approach for immunotherapy. *Hum Gene Ther*. 2002;13:855-866.
64. Molinier-Frenkel V, Lengagne R, Gaden F, et al. Adenovirus hexon protein is a potent adjuvant for activation of a cellular immune response. *J Virol*. 2002;76:127-135.
65. Tang J, Olive M, Champagne K, et al. Adenovirus hexon T-cell epitope is recognized by most adults and is restricted by HLA DP4, the most common class II allele. *Gene Ther*. 2004;11:1408-1415.
66. Crawford-Miksza L, Schnurr DP. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. *J Virol*. 1996;70:1836-1844.
67. Piedra PA, Poveda GA, Ramsey B, McCoy K, Hiatt PW. Incidence and prevalence of neutralizing antibodies to the common adenoviruses in children with cystic fibrosis: implication for gene therapy with adenovirus vectors. *Pediatrics*. 1998;101:1013-1019.
68. Rux JJ, Kuser P, Burnett RM. Structural and phylogenetic analysis of adenovirus hexons by use of high-resolution x-ray crystallographic, molecular modeling, and sequence-based methods. *J Virol*. 2003;77:9553-9566.
69. Rux JJ, Burnett RM. Type-specific epitope locations revealed by X-ray crystallographic study of adenovirus type 5 hexon. *Mol Ther*. 2000;1:18-30.
70. Horwitz MS. *Adenoviruses*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2002;2301-2326.
71. Shenk TE. *Adenoviridae: The Viruses and Their Replication*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2002;2265-2300.
72. Shayakhmetov DM, Lieber A. Dependence of adenovirus infectivity on length of the fiber shaft domain. *J Virol*. 2000;74:10274-10286.
73. Yotnda P, Onishi H, Heslop HE, et al. Efficient infection of primitive hematopoietic stem cells by modified adenovirus. *Gene Ther*. 2001;8:930-937.
74. Busch DH, Pamer EG. T cell affinity maturation by selective expansion during infection. *J Exp Med*. 1999;189:701-709.
75. Haanen J, Wolkers MC, Kruisbeek AM, Schumacher TN. Selective expansion of cross-reactive CD8+ memory T cells by viral variants. *J Exp Med*. 1999;190:1319-1328.
76. Bollard CM, Kuehnle I, Leen A, Rooney CM, Heslop HE. Adoptive immunotherapy for posttransplantation viral infections. *Biol Blood Marrow Transplant*. 2004;10:143-155.
77. Heslop HE, Ng CYC, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nat Med*. 1996;2:551-555.
78. Riddell SR, Walter BA, Gilbert MJ, Greenberg PD. Selective reconstitution of CD8+ cytotoxic T lymphocyte responses in immunodeficient bone marrow transplant recipients by the adoptive transfer of T cells clones. *Bone Marrow Transplant*. 1994;14:S78-S84.
79. Rooney CM, Smith CA, Ng C, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr virus–related lymphoproliferation. *Lancet*. 1995;345:9-13.
80. Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus–induced lymphoma in allogeneic transplant recipients. *Blood*. 1998;92:1549-1555.
81. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med*. 1995;333:1038-1044.
82. Einsele H, Roosnek E, Rufer N, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood*. 2002;99:3916-3922.
83. Kozarsky KF, Wilson JM. Gene therapy: adenovirus vectors. *Curr Opin Genet Dev*. 1993;3:499-503.