Adoptive immunotherapy is an attractive and elegant strategy for treating a variety of life-threatening diseases. Several approaches have been developed to generate antigen-specific CD4+ and CD8+ T cells for adoptive T-cell therapy in cancer and infectious diseases. Currently, many approaches are based on either the use of autologous peptide pulsed dendritic cells as antigen-presenting cells or nonspecific expansion of T-cell clones. Unfortunately, current approaches lack the ability to serve as reproducible and economically viable methods. Several groups are developing new artificial approaches to overcome problems associated with dendritic cells and the nonspecific expansion of T-cell clones in order to make adoptive immunotherapy more feasible and effective. Thus, by increasing the availability of adoptive immunotherapy, we will be able to better determine the efficacy of the approaches in the treatment of a variety of diseases.

In this review, we focus on technological advances that will facilitate adoptive immunotherapy. Specifically, we summarize current strategies which are either based on artificial antigen-presenting cells or on T-cell receptor gene transfer.

Summary
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In this review, we focus on technological advances that will facilitate adoptive immunotherapy. Specifically, we summarize current strategies which are either based on artificial antigen-presenting cells or on T-cell receptor gene transfer.

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Introduction

Over the past 15 years, adoptive T-cell transfer in immunotherapy has become a promising means for the supportive treatment of a variety of infectious diseases and cancer. Adoptive transfer includes the ex vivo stimulation, activation and expansion of autologous antigen-specific T cells over a short time period, followed by infusion back into the patients. This requires a technique for the rapid and reproducible expansion of highly specific cytotoxic T-lymphocyte lines or clones from low precursor frequencies to clinically relevant numbers.

Antigen-specific adoptive immunotherapy was first evaluated in humans using ex vivo expanded cytomegalovirus (CMV)-specific cytotoxic T lymphocytes for the treatment of CMV infection in immunocompromised allogeneic bone marrow transplant recipients (1, 2). While it is common for CMV to cause life-threatening disease in immunocompromised patients, neither CMV viremia nor CMV disease developed in any of the treated patients, indicating that protective T-cell immunity against CMV had been restored (1, 2). Following the success of cytotoxic T-lymphocyte–based adoptive immunotherapy for CMV, ex vivo expanded Epstein-Barr virus (EBV)- and human immunodeficiency virus (HIV)-specific cytotoxic T lymphocytes (38) were also used in treating some EBV-related diseases and aspects of HIV infection.

Rosenberg et al. (9) were the first to expand autologous tumor-specific T cells, referred to as tumor-infiltrating lymphocytes, successfully ex vivo and reinfuse them into melanoma patients. In some patients, they were able to show that reinfused tumor-infiltrating lymphocytes trafficked back to tumor sites and were apparently directly inducing tumor shrinkage, although autoimmune attacks on normal Mart-1–expressing tissue were also detected. More recently, Mackensen et al. (10), as well as Yee et al. (11), have shown that Mart-1–specific T cells survive in the host for several weeks, which they confirmed by tetramer analysis. The Meidenbauer group also showed that the transferred Mart-1–specific cells could be detected preferentially at the site of tumor or metastases.

Currently, in addition to adoptive immunotherapy being evaluated for use in the treatment of infections with viruses such as CMV (1, 2), EBV (36) and HIV (7, 8), its use in other disorders such as malignant melanoma (9, 12, 13), multiple myeloma (14) and EBV-associated lymphoproliferative disorders and tumors (15–19) is also being evaluated.

Current problems in adoptive immunotherapy

While there have been promising results, as described above, adoptive immunotherapy is still in the experimental phase, restricted by the technical limitations and expense associated with current approaches. For example, one current strategy is based on expanding T cells with dendritic cells derived from the host. However, there are a variety of issues related to the use of host dendritic cells. Specifically, they are expensive and require a lot of time to generate and maintain. Furthermore, several leukophereses are required to obtain sufficient numbers of cells from the patient for the in vitro generation of dendritic cells. Notwithstanding the fact that not every patient can tolerate a leukopheresis, the amount and quality of the dendritic cells generated in vitro is highly variable, due to pretreatment with chemotherapeutic and immuno-suppressive drugs and to the disease itself. In patients with multiple myeloma or breast cancer, for example, the tumor affects both the number of dendritic cell precursors and the function of the dendritic cells themselves (20–23). Furthermore, the presentation of a broad spectrum of endogenous peptides by the dendritic cells makes it almost impossible to induce T cells directed at only the desired antigenic specificity; hence, it is a process which is highly variable and very difficult to standardize. Altogether, these are major impediments for the use of dendritic cells as antigen-presenting cells.

In addition to the intrinsic issues associated with dendritic cells, there are also problems related to the expansion of T cells, whether peripheral blood mononuclear cells, tumor-infiltrating lymphocytes or T-cell clones, associated with current techniques. It is often difficult to expand the T cells, tumor-infiltrating lymphocytes or T-cell clones in vitro to sufficient numbers, either due to the stimulation method or to other unknown factors which influence the culture. While expansion of T-cell clones is more standardized and can yield large cell numbers, T-cell clones are often close to the end of their lifespan after in vitro expansion, which reduces their functional capacity after transfer back into the patient.

In light of all of these problems with the use of dendritic cells as antigen-presenting cells, new methodologies that are dendritic cell–independent are also under development.

Approaches to adoptive immunotherapy

Two promising advances have been pursued in recent years to improve adoptive T-cell transfer.
One line of research has focused on the development of a variety of alternative artificial antigen-presenting cells and hence the enhancement of antigen presentation, whereas the second technological advance targets the T cells themselves via T-cell receptor gene transfer and therefore antigen recognition (Table I).

### Nonspecific stimulation with artificial antigen-presenting cells

To overcome the difficulty in obtaining sufficient amounts of antigen-specific cytotoxic lymphocytes, Oelke et al. (25) developed several strategies using bead- and cell-based artificial antigen-presenting cells for the nonspecific expansion of antigen-specific cytotoxic T lymphocytes derived either from tumor-infiltrating lymphocyte cultures or from tetramer-based sorting for enrichment of antigen-specific cytotoxic T lymphocytes. Their initial approach was based on magnetic beads to which they covalently bound anti-CD3 and anti-CD28 monoclonal antibodies (Fig. 1A). While these artificial antigen-presenting cells have been useful for expansion of CD4+ T cells, anti-CD3/anti-CD28 stimulation alone does not support the long-term growth of CD8+ cytotoxic T lymphocytes (24). In addition to the poor expansion rate, CD8+ cells stimulated with anti-CD3 beads lose up to 90% of their antigenic specificity during the 1–2-week expansion (25). Moreover, these limitations cannot be overcome by adding T-cell growth factors like IL-2 to the culture medium. Due to the limitations associated with CD8+ T-cell proliferation, an alternate approach has been the development of an artificial antigen-presenting cell based on the MHC class I–negative leukemia cell line K562, which has been transfected with both co-stimulatory molecules, such as 4-1BB, a member of the TNF family, and the low affinity Fc gamma receptor CD32 (26) (Fig. 1B). Engagement of 4-1BB by 4-1BB, which is expressed on activated CD4+ and CD8+ cells, leads to decreased apoptosis and increased expansion and IL-2 production of the lymphocytes, all of which facilitates T-cell growth. Furthermore, expression of CD32 allows one to bind anti-CD3 and anti-CD28 monoclonal antibodies to stimulate T cells and to gain additional co-stimulatory capacity, respectively. However, while CD8+ T cells can be expanded in this fashion, there is still a critical loss of antigenic specificity associated with this approach.

Therefore, it is evident that there is an important need to design new approaches that facilitate the expansion of CD8+ T cells to high numbers with defined antigenic specificity.

### Antigen-specific stimulation with artificial antigen-presenting cells

The antigenic specificity of the T cells is critical for the efficacy of the adoptively transferred T cells into patients and may also reduce associated side effects, such as autoimmune diseases or graft versus host disease (GVHD). Therefore, many investigators have focused on development of an artificial antigen-presenting cell to facilitate the expansion of antigen-specific T cells.

#### MHC class I–based approaches

Several new approaches to antigen-specific stimulation with artificial antigen-presenting cells have been undertaken. Cell-based approaches were explored by both Sun et al. in 1996 (27) and La-
touche et al. in 2000 (28) using either a Drosophila spp. cell line or a murine system, transduced with a human lymphocyte antigen (HLA) and a variety of co-stimulatory complexes, e.g., B7.1, ICAM-1 and LFA-1 (Fig. 1C). For stimulation of antigen-specific T cells, the cells were co-cultured either with autologous peptide pulsed dendritic cells or with transduced artificial antigen-presenting cells. After one week of co-culture, an average two-fold higher CD8+ cell count in the culture was seen when using the artificial antigen-presenting cells as compared to dendritic cells as stimulator cells. In addition, the cytolytic activity against a specific peptide was up to four-fold higher for the T cells stimulated with artificial antigen-presenting cells.

Tham et al. (29) also described a bead-based system, in which they coupled MHC class I peptide single-chain constructs together with B7.1 and B7.2 as co-stimulatory molecules to the surface of latex microspheres (Fig. 1D). Using such engineered latex microspheres, they were able to show stimulation of already peptide-specific T cells in a transgenic mouse system.

Together, these results show the first evidence for an advantage in terms of both antigen specificity and proliferation capacity when using an artificial
system for generating T cells for future adoptive transfer. Recently, we developed an artificial antigen-presenting cell in which HLA-A2-Ig (Fig. 1E) and an anti-CD28 antibody are coupled to the surface of a cell-sized magnetic bead (25) (Fig. 1F). Using this system, we were able to induce and expand peptide-specific cytotoxic T lymphocytes, directed at clinically relevant antigens such as a peptide from the melanoma self-antigen Mart-1 or the CMVpp65 peptide. With this approach, when starting with 10^6 CD8+ T cells isolated from fresh blood, up to 10^9 T cells with a specificity of greater than 85% were obtained. This represents an over 10^6-fold expansion of antigen-specific T cells in less than two months of stimulation. Furthermore, we could induce antigen-specific cytotoxic T lymphocytes against a subdominant peptide from the cancer testis antigen NY-ESO-1, which recognized and lysed allogenic tumor cells in an antigen-specific fashion.

Additionally, we compared the potential of our peptide-loaded artificial antigen-presenting cells with anti-CD3/anti-CD28 beads to expand already peptide-specific cytotoxic T lymphocytes. In contrast to the loss of specificity seen when antigen-specific cells are expanded with anti-CD3/anti-CD28 beads, we found that the T cells cultured with the HLA-Ig/CD28 beads maintained their antigenic specificity, while their numbers were comparable to the nonspecific expansion.

To summarize, we have studied several cytotoxic T lymphocyte targets with a wide range of affinities and modeled those studies on dendritic cell–mediated cytotoxic T-lymphocyte expansion. Artificial antigen-presenting cell–mediated expansion was simpler, highly reproducible and as good as if not better than the “gold standard” dendritic cell–mediated cytotoxic T-lymphocyte induction and expansion.

Thus, artificial antigen-presenting cells represent a robust versatile technology useful for inducing and expanding antigen-specific cells with varied specificity from multiple donors.

MHC class II–based approaches

There are also preliminary studies using MHC class II–based artificial antigen-presenting cells. In 2000, Prakken et al. (30) generated an artificial antigen-presenting cell based on soluble mouse MHC class II molecules, incorporated into liposomes to mimic the physiological interactions between antigen-presenting cells and T cells (Fig. 1G). The artificial antigen-presenting cells were about 6090 nm in diameter and carried up to 160 MHC molecules on their surface, moving freely within their artificial membrane. These experiments show that these artificial antigen-presenting cells can be used to stimulate ovalbumin-specific T cells, as determined by proliferation assay and IL-2 ELISA.

Recently, Maus et al. (31) designed an MHC class II–based artificial antigen-presenting cell using a magnetic bead to which they bound either directly or via streptavidin MHC monomers or tetramers in combination with anti-CD28 (Fig. 1H). These artificial antigen-presenting cells were able to induce effector function in a system with human influenza-specific T cells, but actual T-cell induction or expansion of antigen-specific T cells was not demonstrated.

Redirecting T-cell specificity

Several groups have chosen an alternative approach to overcome current problems in adoptive immunotherapy. One approach is to clone an antigen-specific T-cell receptor and transfect it into naïve autologous T cells and the other is to express a chimeric protein made by fusing the extracellular domains of a targeting molecule such as an antibody or a receptor with a defined antigenic specificity to CD3-zeta (reviewed in refs. 32 and 33). Deeks et al. (34), for example, used a chimeric CD4-CD3–zeta which can target HIV gp120, then transfected naïve autologous T cells with this chimeric antibody–T-cell receptor complex (Fig. 1I). These cells will be activated against the virus when the chimeric CD4 is used by HIV as a receptor.

In a different approach, Kessels et al. (35) reported a mouse system in which they transduced naïve T cells with an influenza-specific T-cell receptor. Using this approach, they were able to generate virus-specific T cells. These T cells were then able to mediate antitumor immunity in vivo against tumor cells which were transfected with the specific antigen. Although only a small number of transduced T cells were injected, these cells expanded 10^3-fold and led to complete tumor regression. None of the possible autoimmune reactions to normal tissue were detected.

Promising results were also published by Brentjens et al. (36) in 2003, when they transduced human T cells with an artificial chimeric T-cell receptor (Fig. 1J). Upon stimulation through the chimeric
T-cell receptor that targets CD19, a B-cell marker which is expressed by a majority of B-cell-derived lymphomas, chronic lymphocytic leukemias and acute lymphatic leukemias, they were able to show a more than $10^3$-fold proliferation of the CD19-specific T cells over a period of 7 weeks with weekly restimulations. Moreover, the addition of IL-15 to the culture was a crucial factor in terms of proliferation and antigen specificity. Similar results were obtained using T cells from patients with chronic lymphocytic leukemias for the transduction with CD19. These cells retained their ability to efficiently lyse autologous tumor cells in vitro, which shows that the capability of the T cells to act as effector cells in vitro was not impaired even by previous chemotherapy.

Conclusions and perspectives

In summary, major advances in adoptive T-cell therapy have resulted from development of artificial systems that facilitate T-cell expansion. Some of the systems for nonspecific expansion are highly useful for nonantigen-specific T-helper cell expansion and are currently being used in clinical trials for HIV therapy. For example, Levine et al. (37) adoptively transferred up to $3 \times 10^{10}$ activated autologous polyclonal CD4+ T cells. Dose-dependent increases in CD4+ T cells and in the CD4:CD8 ratio were observed. Furthermore, a sustained increase in the fraction of cytokine-secreting T cells, as well as a reduction in the percentage of CD4+CCR5+ cells were noted in vivo, which led to the suggestion of enhanced T-cell function and resistance to HIV infection. Other systems, for example the approach of Albani et al. (unpublished data), provide insights into the basic biophysical aspects of the interaction between T cells and antigen-presenting cells, but so far the use of these systems to induce antigen-specific cytotoxic T lymphocytes for clinical trials has not yet been published.

Approaches developed by Latouche et al. (28) and the magnetic bead–based artificial antigen-presenting cells established by our group have potential clinical relevance. Both approaches look very promising in terms of their ability to induce antigen-specific T cells effectively. T-cell expansion of up to $10^5$ cells and the long-term survival of the T cells in culture as we have reported have not yet been published for the Latouche system. Furthermore, this system requires a new transfection for every new antigen of interest.

On the other hand, a bead-based artificial antigen-presenting cell, coupled with an HLA-Ig fusion protein and a co-stimulatory anti-CD28 antibody, as developed by our group, has the advantage over cell-based systems of being an “off-the-shelf” artificial antigen-presenting cell. It could be made available for large-scale production of antigen-specific T cells for use in clinical trials since this type of artificial antigen-presenting cell meets several pivotal requirements: 1) simple, standardized manufacturing with reproducible results; 2) long shelf half-life, allowing economical manufacture of large amounts of GMP-grade beads; 3) stimulation with beads independent of the state of the patient’s dendritic cells; 4) one-type-fits-all-beads (i.e., the same bead can be loaded with different peptides, adding the possibility of treating a variety of diseases); 5) ease in exchanging or adding in co-stimulatory molecules and/or the ratio of MHC peptide vs. co-stimulation, and in introducing an additional HLA class I peptide and/or HLA class II peptide complex of interest to activate antigen-specific T-helper cells. In this system, we were able to show induction and expansion of antigen-specific T cells over the course of 8 weeks to clinically relevant numbers. Furthermore, a bead-based expansion of T cells is by far not as burdensome for the patient as the generation of autologous dendritic cells.

The high prevalence of HLA-A2 in the Caucasian population (with an incidence in other populations as well) of about 45–50% implies that even with only one HLA-Ig–based artificial antigen-presenting cell, we could target up to half the population. Since we can load it with a variety of different disease-specific peptides, these artificial antigen-presenting cells could be used for many diverse diseases. Due to the nature of the beads, only the loaded peptide is presented on the HLA-A2, which makes this technique controllable and reproducible, both crucial features for future clinical use, where standardized protocols are obligatory. The availability of even only 8–10 different HLA-Ig complexes will allow us to extend the approach to cover over 90% of the population.

A drawback for adoptive transfer of T cells may be the induction of autoimmunity. Autoimmune reactions may occur if the peptide is also expressed in normal tissue, as is the case for the Melan A–derived peptide Mart-1. Some patients treated with Mart-1–specific T cells developed vitiligo or uveitis due to the reaction of T cells to normal Mart-1–ex-
pressing tissue (38). This problem might be overcome in the future by more elaborate therapy regimens. One option could be the use of cytotoxic T lymphocytes which are transduced with the herpes simplex virus tyrosine kinase (HSV-TK), so that a possible autoimmune response could be overcome by ganciclovir treatment (39–41).

When using techniques which utilize gene-modified T cells, the formation of mixed T-cell receptors, consisting of endogenous and exogenous chains, can also lead to undesirable side effects, such as autoimmune disease, and may result in GVHD. Another problem with the use of T-cell receptor gene transfer may be the histocompatibility mismatch between the T-cell receptor donor and the patient. There is a risk that the synthetic T-cell receptor may react against the allo-MHC complexed with self peptide. Chimeric receptors, as used by Latouche’s group, do not undergo rearrangement, but the receptor itself might be immunogenic, since it is a fusion of different proteins to a new molecule. In addition, it is unclear if the signaling properties of this kind of receptor are comparable to those of a normal T-cell receptor. These chimeric receptors may act MHC independently and might be useful in immunotherapy, when presentation of endogenous peptide via the MHC molecules is absent or insufficient.

Overall, the technological advances in the adoptive transfer of T cells have brought remarkable progress to the field of immunotherapy. Techniques for the reliable induction and expansion of antigen-specific T cells in vitro have been developed and are constantly being enhanced. The first promising results from clinical trials have already been reported. Clearly, there is still a great deal of work to be done to improve in vitro techniques as well as the therapy regimens. Nevertheless, it is evident that adoptive immunotherapy has enormous potential to play a strong role in supportive therapy against cancer and viral diseases as part of the fortification of the immune system.

References

Technological advances in adoptive immunotherapy


