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Targeted cytotoxic therapy: adapting a rapidly progressing anti-cancer paradigm for depletion of persistent HIV-infected cell reservoirs

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Abstract

Purpose of review—HIV infected cells persisting in the face of highly active antiretroviral therapy (HAART) are arguably the greatest hurdle to eradication of the virus from the body. Complementary strategies aimed at selective killing of infected cells are described.

Recent findings—Pioneered by research in the cancer field, various approaches are under development for selective killing of HIV-infected cells. These include targeted cytotoxic proteins, adoptive cell therapy, cytocidal virotherapy, and targeted non-biological drug carriers.

Summary—These developmental efforts may provide a critical complement to antiretroviral therapy in efforts to achieve HIV eradication, or a "functional cure" whereby therapy can be stopped without viral rebound.

Keywords

antibody-drug conjugates; radioimmunotherapy; immunotoxin; adoptive cell therapy; chimeric antigen receptor; oncolytic virotherapy; targeted liposome

Introduction

The ability of modern day HAART regimens to deplete viral loads below detectable limits by standard clinical assays has renewed serious interest in the prospect of eradicating HIV from the body, or at least achieving a "functional cure" whereby therapy can be stopped for lengthy periods without re-emergence of viral loads (1) (2) (3). The major hurdle to achieving this "holy grail" of HIV therapy is the invariable persistence of virus-infected cells, even after many years of highly suppressive antiretroviral therapy. The inevitable result is rapid rebound of viremia upon cessation of HAART, often to pre-treatment levels. Most eradication concepts have focused on activation-induced depletion of latently infected memory $CD4^+$ T lymphocytes, as well as intensification of therapy with additional replication inhibitors. A different (though not mutually exclusive) approach involves targeted killing of HIV infected cells. The rationale is based on the fact that antiretroviral drugs, though extremely efficacious for blocking the HIV replication cycle, fail to directly kill cells that are already infected; targeted killing provides precisely this complementary

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activity. The past two decades have witnessed major advances is devising strategies to directly kill tumor cells based on their surface expression of tumor-associated antigens that can be targeted by antibodies linked to cytotoxic payloads; alternatively they can be killed by adoptive cell therapy with natural or genetically modified cytotoxic T lymphocytes (CTLs). These developments in the cancer field have paved the way for applying parallel approaches against viruses including HIV, although the progress is less advanced and faces the major problem of latently infected cells that are "invisible" to the targeted killing modality unless activated (naturally or deliberately) during the treatment period (1) (2) (3). This article describes how targeted killing approaches developed to treat cancer might be applied toward depletion of HIV infected cells persisting in the face of HAART.

The Cancer Paradigm: How Relevant to HIV Infection?

While very similar in concept, several significant differences can be envisaged between anti-cancer and antiviral applications of targeted cell killing. For cancer, the goal is to achieve efficient killing of the offending tumor cells displaying high levels of the target antigen while minimizing potentially harmful killing of normal cells that express the same antigen, albeit at lower levels. Antiviral applications are much less vulnerable to this problem, since the target antigen is virus-encoded and therefore not present on uninfected cells. In counterpoint to this lesser concern for virus-infected versus cancer cells, the former present the added challenge of releasing bursts of infectious virions, thus amplifying the effects of any remaining infected cells beyond simply their continued proliferation. Another important contrast is that targeting solid tumors is complicated by their existence outside the main vascular and lymphatic compartment, whereas hematopoietic malignancies and HIV-infected cells reside primarily in blood and lymphoid tissues. On the other hand, HIV-infected cells persist in various additional tissues and organs, raising concerns about adequate penetration of candidate targeted cytotoxic agents. Finally, in the case of HIV and many other viruses that establish chronic infection, latently infected cells will escape the targeted killing unless awakened during the course of treatment.

A Diversity of Targeted Killing Approaches

The various approaches for selective killing of HIV infected cells differ with respect to both the targeting moiety and the cytotoxic mechanism. In the present discussion, targeting is directed at the viral envelope glycoprotein (Env) expressed on the surface of productively infected cells. The Env-binding moiety is generally based on a monoclonal antibody (mAb) that binds to either the gp120 or gp41 subunits; alternatively a soluble fragment of CD4 (sCD4), the primary HIV receptor, has been used (in a few instances in combination with coreceptor CCR5 or CXCR4). For ease of discussion, the term "immuno-" is used herein to encompass both types of targeting motifs. Recognition of highly conserved epitopes is of course essential for antibody-based strategies; the small but expanding repertoire of broadly reactive mAbs against HIV-1 Env makes several options possible (see citations in (4)). Gene transfer is an essential component for some of the approaches discussed herein, with the present focus restricted to targeted cell killing. Recent review articles [e.g. (5)] have summarized the diverse ways in which gene transfer is being developed for a range of anti-HIV therapeutic strategies.

Targeted cytotoxic proteins

Paul Ehrlich's more than century-old idea of the "magic bullet" (6) conceived of chemical compounds that would target specific pathogenic organisms by virtue of their selective affinities for them (http://nobelprize.org/nobel_prizes/medicine/laureates/1908/ehrlich-bio.html). This strategy was brought to life with the advent of mAbs, which could be armed with various cytotoxic

agents for selective killing of the desired target cells. The numerous promising successes in the cancer field (7) have provided support for applying these approaches against HIV infection.

Antibody-drug conjugates (ADCs, immunoconjugates)—Antibodies chemically linked to low molecular weight cytotoxic drugs offer a means for highly potent targeted cell killing (8). ADC developments in the cancer field have focused not only on the choice of the specific antibody and cytotoxic agent, but also on optimization of linker chemistry and conjugation position and stoichiometry. Gentuzimab ozogamicin (Mylotarg), a humanized anti-CD33 mAb linked to calicheamicin, was the first ADC to be approved by the FDA (in 2000 under the accelerated approval program, for treatment of patients ≥60 years old with relapsing acute myeloid leukemia) (9); however in 2010 the drug was voluntarily withdrawn in the U.S. due to safety concerns and lack of demonstrated efficacy (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm216448.htm). More than a dozen ADCs employing different cytotoxic drugs for various cancers are currently in clinical trials, with several in Phase III. Application to viruses, including HIV, has been very limited thus far. An antibody- doxorubicin conjugate was shown to kill cells infected with a laboratory-adapted HIV-1 isolate, and to protect mice from challenge with an engineered infectious murine retrovirus encoding the corresponding HIV-1 Env (10).

Radioimmunotherapy (RIT)—RIT involves linkage of a cytotoxic radionuclide to an antibody for selective killing of cells expressing the corresponding surface antigen (11). Beneficial effects are believed to result from both "direct hit" and "cross hit" activities. Major efforts to optimize RIT for cancer currently involve engineering the antibody to optimize affinity/avidity, increase clearance from blood and kidney, and enhance uptake by the tumor. The choice of radionuclide is important given marked differences in energy, physical half-life, and efficiency of radiation penetration into soft tissues. Success has been achieved in with the development of RIT for refractory or recurring non-Hodgkins lymphoma using radiolabled mAbs against CD20, i.e. Zevalin ([^{90}Y]-ibritumomab tiuxetan) and Bexxar ([^{131}I]-tositumomab), which were approved by the US Food and Drug Administration in 2002 and 2003, respectively. Clinical advances have been made with other hematological cancers and to a lesser extent with various solid tumors. Recent studies in model systems have demonstrated potential utility against infectious diseases caused by bacteria, fungi, and viruses (12). A recent study examined the effects of RIT in SCID mice injected intrasplenically with HIV-infected PBMC. Intraperitoneal treatment wtih a ^{188}Re-labeled anti-gp41 mAb shortly before or after the infectious challenge resulted in a dramatic reduction in infected cells in the spleen at 72 hr post-infection (13); control antibodies (the same unlabeled mAb or an irrelevant ^{188}Re-labeled mAb) had negligible effects.

Immunotoxins—An immunotoxin is a bifunctional protein containing a targeting moiety, typically an antibody or ligand, linked (chemically or recombinantly) to a cytotoxic protein (14) (15). Binding and internalization into target cells expressing the corresponding antigen (or receptor) leads to highly specific cell killing. Developmental efforts in the cancer field have focused mainly on naturally occurring ribosome-inactivating proteins from bacteria (*Pseudomonas* exotoxin A; diphtheria toxin, anthrax toxin) and plants (ricin, saporin, gelonin, pokeweed antiviral protein). The catalytic nature of the cytotoxic moiety confers extremely high potency and specificity of cell killing. The immunotoxin approach is making important strides against hematological malignancies. Ontak (denileukin diftitox), a fusion protein containing interleukin-2 genetically linked to the effector domains of diphtheria toxin, has been approved by the U.S. Food and Drug administration for treatment of persistent or relapsed CD25-positive cutaneous T-cell lymphoma (16). A phase II clinical trial has demonstrated pronounced activity of BL22, an immunotoxin containing an anti-

CD22 mAb linked to effector domains of *Pseudomonas* exotoxin A, in the treatment of chemoresistant hairy cell leukemia; complete remissions were observed, and increased from 25% to 47% with 1 compared to 2 treatment cycles (17).

A serious limitation in the clinical utility of immunotoxins is their immunogenicity owing to the bacterial- or plant-derived foreign protein domains; this restricts immunotoxin treatment to short periods. An interesting approach to this problem involves identifying the critical B-cell epitopes and removing them mutagenically without compromising bioactivity (18). As an alternative, immunotoxins have been designed wherein the cytotoxic domains, like the targeting antibody or ligand moieties, are human-derived (e.g. granzyme B, ribonucleases, apoptosis-inducing proteins) (19).

Immunotoxins have a long history in the HIV field, with the first reports roughly two decades ago demonstrating potent specific *in vitro* killing by CD4- or mAb-targeted immunotoxins based on *Pseudomonas* exotoxin A (PE), ricin, and diphtheria toxin [see (20) for reference citations]. Enthusiasm for immunotoxins to treat HIV infection declined when Phase I clinical trials of sCD4-PE40 in the early 1990s (pre-HAART era) were halted due to dose-limiting reversible hepatotoxicity (20). However subsequent animal studies indicated that liver toxicity was associated with immunotoxins containing basic targeting moieties; this suggested that the liver toxicity of sCD4-PE40 was probably due to the high isoelectric point (8.86) of the sCD4 moiety. 3B3-PE38, a new PE-based immunotoxin containing a high affinity anti-gp120 single chain variable fragment (scFv) in place of sCD4, proved significantly more potent than sCD4-PE40 and displayed no hepatotoxicity in rheseus macaques [see (21) for reference citations]. Most importantly, studies both in cell culture (22) and in the SCID-hu thy/liv mouse model (23) revealed a critical insight that is highly relevant for the clinical potential of immunotoxin treatment against HIV infection: these agents are minimally effective against a spreading infection when used alone, but they show profound cooperative activity when used in conjunction with HAART drugs. The logical explanation derives from that fact that an immunotoxin cannot kill an infected cell until it begins expressing Env at the cell surface, by which time the infection has begun to spread; without the benefit of a replication inhibitor(s), the immunotoxin alone is insufficient. Conversely, as noted in the Introduction, a replication inhibitor is highly effective at blocking viral spread, but has little effect on cells that are already infected. This is the explanation for the potent formal synergy (mutual potentiation) observed between reverse transcriptase inhibitors and an immunotoxin in cell culture (22). As a consequence, the presence of an immunotoxin during treatment with replication inhibitors profoundly suppressed viral rebound after cessation of all therapy, both in cell culture (22) and in a murine model (23). These findings provide the rationale for combining immunotoxins or other targeted killing strategies with HAART in efforts to deplete persisting reservoirs of infected cells (20) (21).

Adoptive cell therapy with engineered T cells

For the present discussion, the focus is on adoptive transfer of T lymphocytes directed against antigens expressed on the surface of diseased cells as an a means for targeted cell killing (24) (25) (26). Antigenic specificity can be provided by a natural T cell receptor (TCR), which recognizes processed antigenic peptides in an MHC class I-dependent manner. The TCR can be either the native protein complex expressed on donor- or patient-derived CTLs, or a recombinant TCR transduced *ex vivo* into patient cells prior to infusion. An alternative mode of antigen recognition is via a chimeric antigen receptor (CAR, also called a T-body), typically composed of an scFv (or ligand) against the desired surface antigen linked to a suitable transmembrane region and one or more cytoplasmic domains containing signaling motifs critical for CTL effector function and survival. The CAR gene is introduced into patient lymphocytes *ex vivo* by retroviral transduction; the cells are then

expanded and infused back into the patient, typically following a nonmyeloablative lymphodepletion regimen. Unlike a TCR, a CAR binds to the intact antigen expressed on the target cell surface. CAR recognition is thus independent of MHC and the associated requirement for antigen processing by the target cell, thereby providing a means to circumvent tumor and viral immune evasion strategies based on disruption of antigen presentation. CARs also have the potential for more widespread utility compared to TCRs, which must be applied in personalized fashion.

Recent clinical trials have produced some impressive results related to the enhancing *in vivo* durability of CAR-modified cells. For example, persistence of a CAR directed against a neuroblastoma-associated antigen was greatly enhanced by transducing the gene preferentially into CTLs specific for Epstein-Barr virus; the effect was presumably mediated by antigenic stimulation and costimulation of the cells upon engagement of their native virus-specific TCRs in the body (27). In a clinical study of a patient with progressive follicular lymphoma, transduction with a second generation CAR recognizing the B cell antigen CD19 resulted not only in a major regression of the lymphoma, but also complete loss of $CD19^+$ cells from the bone marrow at 36 weeks post-infusion; the CAR transgene was still detectable in PBMC at 27 weeks (28). CAR technology is advancing at a rapid pace (29), with enhancements in the signaling domains to improve survival, cytokine secretion, and target cell lysis by the modified CTLs (30, 31). However two independent CAR trials reported death of a patient, in one case presumably resulting from on-target cytotoxicity against normal cells (32); these unfortunate adverse occurrences raise a cautionary flag for adoptive cell therapy approaches.

Adoptive cell therapy holds a special place in the history of HIV treatment, since it was the basis for the only documented biomedical intervention to achieve long term drug-free control of HIV-1 infection, i.e. by allogeneic transplantation of an infected leukemia patient with hematopoietic stem cells from a donor homozygous for the CCR5 Δ32 allele (33). Indeed major efforts are underway to replicate this phenomenon by *ex vivo* genetic engineering of patient CD4 cells to abolish CCR5 expression, e.g. by genetic excision of the gene using zinc-finger nucleases (34) (35). In applying adoptive cell therapy for targeted killing of HIV infected cells, progress has been made in laboratory studies. Thus peripheral CD8 T cells engineered to express the TCR from an HIV-1 gag-specific CTL clone displayed potent anti-HIV-1 activity *in vitro* and in a murine model (36). Similarly HIV-specific CTLs were generated by TCR transduction of human hematopoietic stem cells (37). The issue of CTL escape by HIV-1 was addressed using phage display to isolate anti-gag TCRs with enhanced affinity coupled with the ability to recognize the escape variants (38). Also, CAR technology has been applied to HIV; clinical development remains at an early stage as demonstrated by the limited virological responses in subjects treated with CAR constructs containing first generation signaling motifs (39) (40) (41); some findings indicated that HIV-specific CD4 helper function might improve the responses.

Other strategies

Genetic engineering allows the design of novel viruses with potential beneficial properties. Oncolytic viruses engineered to selectively infect and kill specific tumor cells have been the subject of much innovative research over the past two decades (42). This concept seems adaptable to the development of cytotoxic virotherapy aimed at pitting the designer virus against pathogenic virus. Indeed engineered virus particles displaying CD4 and CXCR4 have been generated from rabies virus (43), vesicular stomatitis virus (44), and HIV (45) (46); these viruses display the expected targeting to HIV-infected cells. However there has been little advancement beyond these initial demonstrations.

Liposomes represent a type of non-biological carrier for delivery of therapeutic agents. Indeed immunoliposomes targeting cancer cells is a robust field of research (47). The concept is being applied to HIV infection with a variety of encapsidated agents, including cytotoxic drugs (48).

Conclusions

The central point underlying the present discussion is that therapeutic strategies aimed at a functional cure for HIV infection might benefit from the special advantage of augmenting inhibitors of virus replication (i.e. HAART drugs) with selective killing of HIV-infected cells. Of the various approaches summarized herein, immunotoxins and adoptive cell therapy have advanced farthest toward clinical testing in conjunction with HAART. Success will depend not only on attacking the cells that are actively producing virus during the treatment period, but also on depleting the reservoirs of latently infected cells become sensitive only after activation and surface Env expression. Toward these ends, promise is shown in minimizing the immunogenicity of immunotoxin proteins to permit more treatment cycles, as well as in the creative strategies to enhance the *in vivo* survival and activity of adoptively transferred HIV-targeted CTLs. Further clinical development will benefit from the recent establishment of nonhuman primate models in which to probe the mechanisms governing viral persistence in the face of HAART (49) (50). The ever-expanding repertoire of targeting moieties and cytolytic modalities provides hope that biomedical intervention may someday achieve what only a short while ago was considered unthinkable - healthy living after HIV infection without the need for continuous antiretroviral therapy.

Targeted Antiviral Program

- Targeted Antiviral Program. Targeted killing of infected cells represents a critical complement to HAART for eradicating persisting HIV infection.
- Pioneered by progress in the cancer field, promising approaches include targeted cytotoxic proteins, adoptive cell transfer, engineered cytolytic viruses, and immunoliposomes carrying cytotoxic drugs.
- Immunotoxins and engineered CTL against HIV have received considerable attention.

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observed viral persistence in blood and various lymphoid tissues despite strong drug-mediated suppression parallels the well known features HIV-1 persistence in HAART-treated humans. These findings suggest that the RT-SHIV/rhesus system will be an excellent model in which to study viral reservoirs and approaches to their eradicatiaon.