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Immunology and Gene Therapy: Shoulder to Shoulder Into the Fray

International Symposium: Critical Frontiers Between Immunity and Gene Therapy

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Functions of the immune system are critical for the outcome of gene therapy. Immunologists, virologists, and gene therapists met in Pamplona, Spain, in October 2009 to present and analyze common frontiers in these fields under the auspices of the Ramón Areces Foundation (http://www.fundacionareces.es). Antibodies and cell-mediated immune mechanisms can be either friends or foes in gene therapy. A form of Murphy’s law seems to apply: “If an immune response is to be induced, it will usually be weak. If an immune response is to be avoided, it will be almost always neutralizing.”

Transfer of therapeutic genes is most efficient when delivered by viral vectors, but the greatest obstacle to the use of such viral vectors is their strong immunogenicity. In this regard, neutralizing antibodies can preclude re-administration of the vector, and cellular responses frequently extinguish the expression of the foreign transgene. On the other hand, the destructive mechanisms of the immune system are frequently exploited by gene therapy approaches to vaccinate against microbial pathogens or destroy cancer cells.

Immunity to vectors

Katherine High (Children’s Hospital, Philadelphia, PA) presented data on a hemophilia B trial using recombinant adeno-associated viral (AAV) vectors expressing clotting factor IX. Initial efficacy data in the first patient receiving the highest vector dose were highly encouraging. However, although the therapeutic vector was able to correct the disease in hemophilic dogs for more than 8 years, it ultimately failed to work in patients. The discrepancy resides in the cellular immune response against a vector that naturally infects humans but not dogs. Studies revealed that CD8+ T cells directed against vector capsid proteins eliminated transduced hepatocytes. Using T cells from patients, several experimental tools have been generated to study the immunogenicity of the vector capsid, including a soluble T-cell receptor (TCR) tetramer that detects those cells that are presenting the antigen. The workers are testing downregulation of the major histocompatibility complex class I antigen presentation pathway using the proteasome inhibitor bortezomib preclinically, as well as exploring the use of classic pharmacological immunosuppressants to avoid the loss of therapeutic gene expression.

In the same line, Ignacio Melero (Center for Applied Medical Research (CIMA), Pamplona, Spain) showed that liver re-expression of a reporter transgene visualized by positron emission tomography is possible upon four repeated intravenous injections of first-generation adenovirus in pharmacologically immunosuppressed macaques. For this purpose, immunosuppressant cocktails included the anti-CD20 monoclonal antibody rituximab, which depletes B cells. There was good correlation between the inhibition of the anticapsid humoral and cellular immune responses and successful transgene reexpression. Katherine High and Ignacio Melero agreed that in nonhuman primates being treated with immunosuppressants, there seemed to be partial attenuation of the efficiency of gene transfer following the first administration of the vector, whether using adenovirus or AAV vectors.

Viruses have developed strategies to hide themselves from the immune system, and learning their tricks might lead to a way to escape an immune reaction following gene transfer. In this regard, Antonio Alcamí (Centre for Molecular Biology, Madrid, Spain) presented interesting studies examining virally encoded proteins from herpesviruses that are able to counteract the actions of several chemokines. The gG protein from herpes simplex virus (HSV) 1 and 2 has been identified as a factor that promiscuously neutralizes multiple human chemokines, including CXCL-10 (IP10), which is critical for cellular immune responses.

Gene therapy for noninfectious and nonmalignant diseases

Classically, most gene therapy trials have targeted cancer or chronic infections. However, most remarkable successes at the present time are being achieved in diseases in which limited correction of a gene defect meaningfully modifies the course of the disease. These illnesses include the primary immunodeficiencies and inherited diseases of the retina.

Ex vivo gene therapy, when feasible, bypasses some of the obstacles posed by the immune system to achieve sustained transgene expression. Bone marrow stem cells are attractive targets to be manipulated ex vivo. Juan Bueren (Centro de
Investigaciones Energéticas, Medioambientales, y Tecnológicas, Madrid, Spain) showed correction of hematopoietic progenitors from patients with Fanconi’s anemia (FA), which is a rare recessive autosomal and X-linked chromosomal instability disorder that usually leads to bone marrow failure and leukemia. Because allogeneic transplantation leads to significant risks of morbidity and mortality, particularly in transplants from unrelated donors, there is a great need for new therapies to treat these patients. In contrast to successful results obtained in the gene therapy of genetic immunodeficiencies, attempts to treat FA by correcting CD34+ cells with gammaretroviral vectors have failed.

Juan Bueren’s group has recently proposed that the use of total bone marrow cells corrected with lentiviral vectors, instead of CD34+ cells transduced with gammaretroviral vectors, might be an improved approach to facilitating the engraftment of FA patients with corrected hematopoietic stem cells. Because the best moment for harvesting FA bone marrow grafts would be early after the diagnosis of the disease, they propose the storage of frozen autologous bone marrow cells that could be transduced and transplanted into patients upon the onset of hematological problems. Using a state-of-the-art approach, they have also shown that induced pluripotent stem cell technology can be used to generate disease-free hematopoietic progenitors from the skin of FA patients. However, this latter technology is still far from the clinic, and improvements in the differentiation and purging of undifferentiated induced pluripotent stem cells are still needed. Nevertheless, these approaches offer new hope for treating a disease that is unusually frequent in the Spanish Gypsy population, in which 1 of 60 individuals is a carrier of an ancestral FA mutation.

**Viruses to induce and sustain prophylactic and therapeutic immune responses**

Ramón Alemany (Instituto Catalán de Oncología, Barcelona, Spain) commented that during viral oncolysis the immune system is often responsible for the destruction of tumor cells, whereas the virus acts only to spark an immune response against tumor antigens; this could be considered an “immunocentric” point of view. In contrast, from a “virocentric” perspective, the virus should infect and kill all tumor cells, and for that purpose the immune system represents an obstacle. An example of immunocentricity was presented by Richard Vile (Mayo Clinic, Rochester, MN), who showed that the antitumoral effect provided by an oncolytic virus such as vesicular stomatitis virus was mediated by an acute innate antiviral immune response. The fact that this response was at the same time responsible for the elimination of the virus shows an intricate balance among safety, restriction of oncolysis, and significant immune-mediated antitumor effects. Examples of the virocentric point of view were presented by Ramón Alemany, who showed that oncolytic adenoviruses armed with fusogenic or matrix-degrading transgenes can facilitate the spreading of viruses inside tumors. But whether the mechanisms are “immunocentric” or “virocentric,” delivery of the virus to metastatic tumors constitutes a bottleneck that needs to be resolved.

An original approach was presented by Richard Vile, who showed that vascular permeability to circulating reovirus can be increased by the use of vascular endothelial growth factor (VEGF). This strategy increased virus replication in endothelial cells close to or inside the tumor, providing a local source of virus and ultimately leading to vascular collapse. VEGF is not likely to be an acceptable agent for cancer therapy, and Dr. Vile showed that hypersensitivity to it can be attained by abrupt cessation of anti-VEGF monoclonal antibody therapy. Delivery of virus to tumors using circulating carrier T and dendritic cells (DCs) is another interesting strategy. However, a low viral multiplicity of infection should be used to load these cells so as to avoid blocking delivery by neutralizing antibodies.

Tumor targeting can also be achieved by using tumour-specific promoters. In this regard Cristian Smerdou (CIMA, Pamplona, Spain) proposed the use of a hybrid adenovirus vector carrying the sequence of a Semliki Forest virus (SFV) replicon expressing interleukin-12 (IL-12). In this vector, the SFV replicon was under the transcriptional control of the α-fetoprotein promoter, leading to high expression of IL-12 and SFV-elicited apoptosis exclusively in hepatocellular carcinoma cells. The approach achieved good antitumoral responses in rats, with virtually no toxicity.

IL-12 is a potent antitumoral and antiviral cytokine that was revisited in this meeting by several groups. Results obtained with new and more potent viral vectors expressing IL-12, such as SFV or inducible gutless adenovirus, were presented in clinically relevant disease models. In particular, Gloria Gonzalez-Aseguinolaza (CIMA, Pamplona, Spain) showed that chronic infections with woodchuck hepatitis virus (WHV)—a hepatitis virus that induces a disease very similar to hepatitis B in woodchucks—could be completely resolved when treated intrahepatically with a gutless adenovirus vector expressing IL-12 in a mifepristone-inducible fashion. However, this dramatic effect was observed only in woodchucks that had a low or intermediate WHV viremia, apparently as a result of an intriguing lack of responsiveness of lymphocytes from animals with a high viral load. Preliminary results indicate that this tolerance could be blocked by a synthetic peptide that inhibits transforming growth factor-β (TGFβ) activity, suggesting that a combined therapy with the gutless adenovirus IL-12 vector and TGFβ neutralization could be efficacious in animals with high viremia.

Woodchucks infected with WHV constitute another excellent model for cancer therapy in that these animals develop spontaneous hepatocellular carcinoma that closely resembles the natural evolution in patients. Cristian Smerdou showed that treatment of these tumors with SFV expressing IL-12 was able to induce transient tumor regressions with reductions in tumor volume of up to 80%. However, tumors were able to regain growth a few weeks after vector administration.

An interesting new concept for inducing antitumoral immune responses is based on the induction of autoimmunity to normal tissues from which a tumor has developed. Richard Vile showed that pathogen-like cytotoxicity to a normal tissue induced by expressing vesicular stomatitis virus G protein in association with the immune adjuvant gene hsp70 generates anti-self responses to normal tissue antigens that are sufficient to induce tumor rejection. This strategy could be a double-edged sword because of the high toxicity of autoimmune responses. However, for tissues such as the pancreas it is possible to decouple antitumor immunity from autoimmune responses by depleting regulatory
T cells before the induction of inflammatory killing of the normal exocrine pancreas.

Induction of antitumor immune responses in the brain is an extremely difficult task because of the immunoprivileged status of this organ. Pedro Lowenstein (University of California, Los Angeles, Los Angeles, CA) was able to overcome brain immunoprivilege by genetically modifying the tumor in intracranial glioblastoma-bearing rodents. In his approach, two first-generation adenoviruses encoding sFLT-3L and HSV-tk were used. Selective killing of tumor cells with the thymidine kinase metabolite ganciclovir and local attraction or differentiation of DCs with sFLT-3L synergized to induce cutaneous responses. These immune responses were mediated by cytotoxic T lymphocytes (CTLs), and critical roles were defined for interferon-γ (IFN-γ) release at the immunological synapse and for TLR2 stimulation by dying tumor cells. Sterile stimulation of TLR2 takes place by means of release of the HMGB1 nuclear protein. The strategy is entering clinical trials for a brain tumor type with a dismal prognosis.

David Sancho (Cancer Research UK, London, UK), from Caetano Reis e Sousa’s group, presented work on the C-type lectin CLEC9A that is expressed by a restricted subset of DCs. This is the main subset responsible for CTL cross-priming. CLEC9A seems to be a marker in human peripheral blood for the functional equivalent of the CD8α dendritic subset in mice. Antibodies directed to CLEC9A can be used to target tumor antigens for their cross-presentation to CTLs, achieving tumor eradication in mice. The natural ligand for CLEC9A is still unknown, but it seems to be a cytoplasmic protein that is exposed upon cell death. CLEC9A couples necrosis recognition to activation by DCs of the Syk tyrosine kinase and regulates cross-priming to dead cell–associated antigen. Elucidation of how to efficiently induce CTL cross-priming can offer abundant opportunities for prophylactic and therapeutic vaccination.

Genetic vaccines

Despite the fact that the immunogenicity of viral vectors can hinder their use in gene therapy applications, this property makes them excellent candidates for genetic vaccination. In this context, the use of viral vectors to vaccinate against diseases produced by highly pathogenic human viruses such as HIV and severe acute respiratory syndrome (SARS) was discussed.

Numerous vaccines for HIV have been developed, but thus far clinical trials have failed to show any efficacy against HIV infection. Even results with a preventive vaccine based on recombinant adenoviruses seemed to favor HIV transmission, particularly in adenovirus-immune vaccinees. However, recently a vaccination clinical trial in Thailand using recombinant canarypox vector vaccine (ALVAC-HIV) plus two booster injections of recombinant glycoprotein 120 provided preliminary evidence that an HIV vaccine regimen has the potential to prevent a small percentage of infections and has brought poxvirus-derived vectors to the forefront. Two of the most promising poxvirus vectors are the highly attenuated modified vaccinia virus Ankara (MVA) and the modified Copenhagen strain NYVAC. Mariano Esteban (Centro Nacional de Biotecnologia, Madrid, Spain), a member of the EUVACOV (a European consortium working on the development of new HIV vaccines), showed data indicating that both viruses deserve consideration as future HIV vaccines as supported by immune correlates and molecular requirements. In macaques, excellent protection against SIV correlated with intense immune responses, and these results have led to NYVAC and MVA candidate vectors that have entered phase I clinical trials for both HIV subtypes, clades B and C.

Jay Berzofsky (National Institutes of Health, Bethesda, MD) presented work that showed the important role of IL-15 in the development of high-avidity cytotoxic T cells, which are also influenced by the adjuvant effect of TLR9, TLR3, and TLR2 agonists. His data showed that IL-15 or the triple combination of TLR agonists does not increase the number of responding T cells but improves quality by inducing T cells of greater functional avidity. Importantly, an intrarectal vaccine incorporating IL-15 and TLR agonists given to macaques very efficiently induced resistance to intrarectal SIV infection. Berzofsky also stressed the importance of induction of T-cell immune responses in the target tissues known to be the entry port for the virus. In particular, he showed that mucosal immunity is necessary for protection against mucosal viral infection. He also explained the value of blocking negative regulatory elements such as regulatory type II natural killer T cells, regulatory T cells, TGF-β, or PD-1 to maximize vaccine efficacy. The importance of IL-15 was also underscored by Ignacio Melero, who showed that sustained liver production of this cytokine helps activation of memory CD8+ T cells, natural killer cells, and interferon-producing killer dendritic cells with therapeutic potential for transplanted tumors. In addition, he showed that IFN-α-derived signals are critical for naive T cells to acquire cell-killing capability.

Luís Enjuanes (Centro Nacional de Biotecnología, Madrid, Spain) showed that the elimination of a single gene from the SARS coronavirus genome (gene E) produced an attenuated virus able to protect against SARS in animal models for this disease. This attenuated virus seems to be more cytopathic than the wild-type SARS-coronavirus and apparently can increase the expression of cellular stress genes in infected cells, contributing to the generation of stronger antiviral immune responses. Developing vaccines for a deadly virus such as SARS seems to be an achievable task with this attenuated virus.

Ex vivo engineering T cells for anticancer therapy

T cells are endowed with a combinatorial repertoire of antigen receptors (TCRs). The repertoire is biased in such a way that self-reactivity is diminished, and those remaining lymphocytes reacting to self-antigens are typically of low TCR avidity. Therefore, in cancer and chronically infected patients high-avidity TCRs are frequently absent. Adoptive T-cell therapy strategies have been devised to avoid these problems.

Chimeric antigen receptors (CARs) have been constructed that encompass an extracellular single-chain antibody and intracellular signaling molecules of the TCR-CD3ζ signaling machinery or the cytoplasmic domains of T-cell costimulatory molecules such as CD28, 4-1BB, or OX40. Gianpietro Dotti (Baylor College of Medicine, Houston, TX) reported his work in collaboration with Malcolm Brenner on an anti-CD19 CAR. This receptor recognizes all B cells (malignant or not) and is fused to the signaling cytoplasmic domain of CD3ζ. The construct was engineered in a clinical-grade retrovirus and shown to be effective in transducing human T cells. Anti-CD19-CAR-modified T cells showed cytotoxic activity against human CD19-positive
lymphomas in vitro. However, these T cells had limited persistence in severe combined immunodeficient mice grafted with human B-cell lymphomas. The problem was circumvented by generating a CAR that also encoded the CD28 costimulatory endodomain. Comparative experiments to determine the optimal costimulatory molecule are ongoing in xenograft models.

A phase I clinical trial for patients with B-cell lymphomas was also presented. These patients received anti-CD19-CAR T cells, and preliminary data suggest that T cells transduced with anti-CD19 CAR that also encodes the CD28 endodomain have longer persistence than T cells expressing the same CAR that lacks the CD28 endodomain. Baylor’s group is also expressing the same CAR that lacks the CD28 endodomain in mouse models. As a safety measure, to prevent uncontrolled proliferation of T cells, a suicide gene has been devised to prevent uncontrolled proliferation of T cells transfected with high-avidity TCRs. Treatment of melanoma patients with ex vivo–expanded tumor-infiltrating lymphocytes allowed the identification of CD8+ clones with very high avidity for the MART-1 peptide, and TCRs from these clones were cloned and expressed. The chains of the TCR were introduced into clinical-grade retrovirus and used to engineer total proliferating T cells from peripheral blood. Twenty patients were preconditioned with chemotherapy and total body irradiation and then transferred with engineered T cells plus IL-2, resulting in impressive clinical responses in six patients with metastatic melanoma. Another high-avidity TCR was prepared that recognizes g100-HLA-A2. A trial using this TCR with a similar approach included 16 patients with three clinical responses. Transient skin rashes, vitiligo, a case of autoimmune deafness, and a resolving case of uveitis were reported and attributed to melanocyte destruction. Further improvements are envisioned as a result of using younger T-cell cultures with more capacity to expand and persist, by developing higher-avidity TCRs in mice transgenic for HLA-A2 and stressing the need for polyfunctional effector CD8+ and CD4+ T cells. Preconditioning to eliminate competition and/or suppression by endogenous T cells seems to be critical.

In the context of allogeneic bone marrow transplantation, the donor immune system can damage the host tissues in what is called graft-versus-host disease (GVHD). High-avidity allogeneic T cells mediate damage but can also destroy malignant cells. Donor lymphocyte infusions are often an efficacious strategy to fight posttransplant relapses from the hematological malignancy. However, this increases the risk of severe acute GVHD. Claudio Bordignon (MolMed, Milan, Italy) presented a system for rendering safer infused T cells that is based on transfection with a retrovirus encoding the suicide gene HSV-1k and a truncated form of nerve growth factor receptor as a surface marker to permit immune selection of transfected lymphocytes. Dr. Bordignon described a phase II clinical trial with 54 patients at high risk of posttransplant relapse. Twenty-eight patients needed donor lymphocyte infusion, and in 10 patients with acute GVHD, ganciclovir-mediated elimination of thymidine-kinase-transfected lymphocytes was curative. An interesting observation was the genomic loss of the recipient’s mismatched HLA haplotype in the leukemic cells of a patient who had undergone transplantation of haploidentical hematopoietic stem cells. This phenomenon could represent a mechanism of tumor escape from the selective pressure of a patient-specific graft-versus-leukemia reaction.

**Gene therapy and immunotherapy getting translational hand in hand**

A prominent feature of current frontiers in gene therapy and immunology is a commitment to translational research. Indeed, many methods to foster, harness, or repress immunity in gene therapy are becoming translational at a fast pace. It is clear that the efforts of immunologists and gene therapists must go together in order to craft wisely at the laboratory bench what is to be tested in patients. How to elegantly dodge the immune system, as bullfighters do with bulls, is a topic that could be properly addressed in a meeting held in Pamplona, where creative discussions and ideas were fostered by excellent Navarra tapas and wines to prove that Mr. Murphy sometimes can be wrong.

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