Long-Term Follow-Up Of Gene Transfer Patients

31st Biological Response Modifiers Advisory Committee Meeting

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Holiday Inn
2 Montgomery Village Ave.
Gaithersburg, MD

1. Introduction

Previous meetings of the Biologicals Response Modifiers Advisory Committee (BRMAC) on November 16-17, 2000 and April 5-6, 2001 involved discussions about long-term follow-up (LTFU)[1] of participants in gene therapy clinical research. This document summarizes those discussions, highlighting where there was not consensus or where outstanding issues still remain.[2] In addition, it provides background pertaining to actual and/or hypothetical long-term risks of malignancies and/or hematologic, neurologic, or autoimmune diseases that may occur to participants in gene transfer trials. With this background, it is the agency's hope that further progress can be made in addressing deficiencies with current guidance for LTFU and to develop principles that could serve as a basis for new guidance.

2. BRMAC November 16-17, 2000 Meeting Summary

The committee generally agreed that LTFU of participants in gene transfer clinical trials is important and efforts to gather data pertaining to the long-term risks of exposure are necessary.

For the purposes of discussion, the committee suggested vectors used in gene transfer research could be divided into classes based on risk factors (table 2). Vector characteristics of particular concern included:

1. The potential to integrate;
2. The potential to replicate;
3. Altered tropisms; and
4. Long latency.

2.1. Table 2: Vector Class and Characteristics

Vector Class	Integration Potential	Replicating/ Defective	Latency Potential
Retroviral Vector	Reliably high	Defective	High
Adeno-associated Virus Vector	Varies, depending on tissue	Defective	Varies
Herpesvirus Vector	None reported	Replicating or Defective	High
Plasmid	Low, may vary depending on method	None	None
Adenovirus Vector	Low	Replicating or Defective	None
Poxvirus Vector	None reported	Replicating	None

[1] For the purpose of discussion, "long-term follow-up"(LTFU) is defined as the follow-up of study participants that occurs beyond the first year after final treatment on protocol. Clinical concerns restricted to an individualized or specific vector or study reagent for a given study would be addressed in the study protocol and would not be material to any guidance on "long-term follow-up".

[2] BRMAC briefing materials for Session III of November 16-17, 2000 and Session III of April 4-6, 2001 meetings summarize the proposed basic framework for future LTFU guidance. Transcripts are available at http://www.fda.gov/ohrms/dockets/ac/cber00.htm

Some vector characteristics were thought to pose higher degrees of long-term risk. For example, integrating vectors have the potential to initiate neoplastic processes depending upon the site of integration, presence of strong promoter/enhancer elements present in the gene transfer vector, and so on. Host characteristics were also discussed and felt to be influential factors. Host characteristics that were discussed included:

1. The immune status of recipient;
2. The route of administration (e.g., intra-venous, intra-arterial, subcutaneous, etc.); and,
3. The type of cell targeted for transformation (e.g., ex-vivo transformation of stem cell, cells capable of division and lasting life cycle vs. irradiated cells, etc…).

3. BRMAC April 5-6, 2001 Meeting Summary

Prior to the April meeting, CBER staff formulated and proposed a framework for future LTFU guidance based on the November discussions.

A three-tier LTFU proposal was based on the committee's advice that vector properties rather than vector types should define the minimum level of clinical information required for adequate long-term follow-up (Table 3). The type of clinical information collected would be driven by perceived long-term risks to participants. The proposal assumed that LTFU of participants in gene transfer clinical trials would be the responsibility of IND sponsors who would report the data to FDA. FDA would maintain a database, and periodically review the database for trends, particularly trends in adverse events.

3.1. Table 3. Proposed Three-Tier System

Tier	Vector Characteristics	Study Participant Follow-up (Past 1st Year)
1	Ex vivo gene transfer with non-replicating vector into cells with demonstrated limited survival of ≤2 weeks in vivo	None
2	All other gene transfer products that are not in tiers 1 or 3	During enrollment, subject education re need for LTFU 1-20 years: data collection by sponsor
3	Replicating or potential to replicate, (except poxvirus and adenovirus) High integration potential Altered receptor tropism Latency potential	During enrollment, subject education re need for LTFU 1-5 years: annual physical exam an medical history by treatment center, and obtain appropriate samples for archive 6-20 years: data collection by sponsor

3.2. Tier 1

Pursuant to the November 2000 meeting, the agency proposed that one group of vectors (Tier 1) could be exempt from the need for long-term follow-up past the first year post-treatment. Products appropriate for Tier 1 would include *ex vivo* gene transfer into cells when all the following conditions are met: 1) cells are no longer replicating or able to survive past two weeks (*i.e.*, irradiated cells), 2) the gene transfer vector is a non-replicating vector, and 3) the gene transfer vector does not

have the potential for contamination with a replicating virus. In order for a gene transfer product to qualify for tier 1, the sponsor would have had to provide data demonstrating the limited survival of the cells in an animal model.

During the 4/6/01 meeting, the Committee reconsidered its earlier recommendation that no LTFU would be needed for some classes of vectors. The majority instead recommended that all gene transfer studies incorporate some form of LTFU. The data collection for Tier 1 could be similar to that proposed for Tier 2, (i.e. clinical data without specimen collection), however the duration of follow-up could be shorter. However, there was not complete consensus on this issue. Some members were unchanged in their view that a vector category exempt from all LTFU studies was justified and desirable.

3.3. Tier 2

The proposed second tier would include vectors and cells intermediate between Tiers 1 and 3 regarding level of risk. For example, poxvirus and adenovirus vectors would be included in Tier 2 because of lack of evidence for persistence or latency. Clinical protocols using cells known to have a long life-span or replication potential exposed *ex vivo* to gene transfer vectors in tier 2 would also qualify for tier 2 LTFU.

Clinical trial participants treated with gene transfer products in tier 2 would be subject to protocol-specific follow-up during the first year, including, at a minimum, a baseline sample of serum and peripheral blood mononuclear cells (PBMC) for archiving. During enrollment, study participants should be educated as to the need to participate in long-term follow-up, for at least 20 years post-treatment. During the period from years 1-20 post-treatment, the sponsor would collect updated subject information (described in more detail below) and report some of the data to the FDA in annual reports.

BRMAC debated the merits of a 20-year follow-up period during the 4/6/01 meeting. Considering the costs and burden of gathering data over a long period, the Committee deliberated about an appropriate follow-up time period for risks relating to rheumatologic/autoimmune, hematologic, infections and latency, malignancy, and neurologic diseases. Some members suggested that autoimmune diseases would appear within the first 5 years and unlikely to present later, hence, it was suggested that the follow-up period be limited when other risks such as malignancy are not primary concerns. Others questioned whether such a limit would be reasonable considering the lack of clinical data to directly support such position.

3.4. Tier 3

Clinical protocols using gene transfer products with one or more of the following characteristics would be placed in tier 3:

1. Replication-competent or potential to replicate (with the exception of poxvirus and adenovirus vectors, see above for explanation),
2. High integration potential, altered receptor tropism, and

3. Potential for latency followed by reactivation.

The study participants treated with gene transfer products in this category would be subject to protocol-specific follow-up during the first year, including, at a minimum, a baseline sample of serum and PBMC obtained for archiving. During enrollment, subjects should be educated as to the need to participate in long-term follow-up for at least 20 years post-treatment. Tier 3 is differs from Tier 2 in that there is more intensive follow up during the first 5 years post-treatment. Study participants who are exposed to tier 3 gene transfer products would be expected to have an annual physical examination years 1-5 at the treatment center at which time appropriate samples would be obtained for archiving. During the next 6-20 year post-treatment, the sponsor would collect updated subject information (described in more detail below) and report some of the data to the FDA in annual reports.

BRMAC generally agreed that limited laboratory sampling and clinical follow-up was necessary for vectors products in Tier 3. However, some members were not comfortable with the suggested 20-year period. Some members questioned whether or not such a database can be achieved (practical consideration) and whether or not there are sufficient concerns to warrant such undertaking (risk-benefit consideration, scientific/medical rationale).

4. LTFU Data Collection and Duration: Considerations:

CBER suggested that a population-based LTFU of gene transfer study participants that allows for the detection of rare clinical events over a 20-year period is desirable especially for associated malignancy risks. A 20-year monitoring program provides an advantage for the detection of rare events that occur years, sometimes decades following protocol therapy. For example, the excess risk of leukemia attributable to treatment for Hodgkin's disease peaks 5 to 9 years following initial therapy and reaches a plateau after 15 years. The relative risk of lung cancer increases steadily with increasing follow-up time and the risk for breast and thyroid cancer does not become apparent until after 10-15 years of observation. If autoimmune, hematologic, neurologic, or oncologic risks were to occur decades following the treatment with a gene transfer product, shorter follow-up periods may not be adequate to detect an incidence above population background.

BRMAC reasoned that practical considerations must be considered when defining LTFU periods. A risk-benefit analysis must be considered before an arbitrary period of 20-years is mandated. Practical barriers to long term follow-up may include 1) lack of adequate research funding for the long term follow-up of subjects 2) clinical follow-up is difficult to perform (patients lost to follow-up, incomplete information, lack of cooperation by treating MD, etc…), 3) autopsies are difficult to obtain (lack of notification when a subject dies, family refuses, etc…), 4) the clinical relevance of data collection is not obvious to investigators or study participants and difficult to motivate participation (will the information collected allows for the advancement of the field, how will this data be used, etc…), and finally 5) this is an unusual commitment on the part of investigators, hosting institutions, and/or trial sponsors.

I was generally felt that identifying and focusing on the most important data would help improve compliance. It was agreed that CBER would further assess the risks factors, associated risks, and time course at manifestation for further discussion with BRMAC.

5. FDA Working Group

CBER convened a LTFU Gene Therapy Working Group to further define clinical concerns related to gene transfer studies. Members were asked to discuss the potential value of long term follow up and to help define the clinical concerns that would justify long term follow-up requirement taking into consideration various aspects of gene transfer studies that could affect long-term outcome and risks to study participant:

1. Level of vector integration, replication, or segregation (e.g., episomes)
2. Duration of gene product expression;
3. In vivo vs. ex vivo transfection
4. Mode of administration (i.v., intra-arterial, intraperitoneal, intraplacental, inhaled, intratrachael, subcutaneous, intra-tumor, etc)
5. Targeted tissue (hematologic stem cells, brain cells, fetal tissue, tumor cell lines, hepatocytes; myocytes, etc…)
6. Transfection of dividing and non-dividing cells
7. Patient-specific factors (immunocompromised host, pediatric population)

The following table identifies the members of the working group:

5.1. Table3: FDA Working Group

Name	Division	Area of Expertise/Role
Philippe Bishop, MD	OTRR/DCTDA/Oncology	Oncology
Patricia Keegan, MD	OTRR/DCTDA	Oncology/Hematology/Deputy Director
Harvey Luksenburg, MD	OTRR/DCTDA/Oncology	Hematology
Anne Pilaro, Ph.D.	OTRR/DCTDA/Clin. Pharm.	Toxicology
Cindy Rask, MD	OTRR/DCTDA/Medicine	Neurology
Steve Rosenthal, MD	OVRR/DVRPA/VCTB	Vaccines/Epidemiology
Stephanie Simek, Ph.D.	OTRR/DCGT	FDA RAC Liaison
Joseph Temenak, Ph.D.	OVRR/DVRPA	Vaccines/Epidemiology
Mark Thornton, MD	OTRR/DCTDA/Immunology	Immunology
Carolyn Wilson, Ph.D.	OTRR/DCGT	Virology

6. Establishing A Database For Epidemiologic Studies

The majority of data from patient exposure to gene transfer product occurs in studies without a control group. Consequently, establishing an epidemiologic database that can be used to determine whether rare and delayed adverse events are occurring at rates above those expected in similar patients population not exposed to the study drug requires careful consideration.

One method to determine whether or not gene transfer products may cause adverse clinical events is to perform a prospective cohort study in which subjects receiving gene therapy are followed over time and compared with another group of individuals not exposed to gene transfer therapy. Prospective cohort studies enables one to determine the incidence and relative risk of outcomes in persons exposed to a risk factor, to investigate multiple outcomes, minimize bias, and study the sequence of events of disease. In determining the long-term health effects of a given exposure in which multiple outcomes are of interest, prospective cohort studies are the studies of choice.

Selection of comparison groups might be difficult. Commonly, a comparison is made with population rates. The observed number of cases in the gene therapy subjects can be compared to the expected number, which is estimated from age/sex adjusted population rates. However, population rates may not be available for populations with the underlying disease.

Another option would be to select and follow a comparison cohort that is similar to the subject cohort. An ideal comparison would be to choose patients with the same underlying disease that were not treated with gene therapy. This type of cohort is not readily available outside of clinical trials.

For outcomes that are rare without gene therapy exposure, unless the relative risk associated with exposure is very large, the cohort approach may not be of value. In general, ad hoc post-marketing cohort studies that do not have control groups usually provide little new information although costs are significant.

The following table outlines samples size needed to detect pre-specified adverse events for both the exposed group and the control group, with alpha=0.05, power=80%.

	Relative Risk to Detect						
Incidence in control group	1.25	1.5	2.0	2.5	3.0	3.5	4.0
0.005	56210	15604	4675	2421	1554	1117	861
0.01	27946	7752	2319	1199	769	552	425
0.05	4204	5334	435	222	141	100	76
0.1	2508	686	200	100	62	43	32
0.15	1566	425	121	59	36	24	17
0.20	1095	294	82	39	15	15	10

Despite the difficulties of conducting pharmacoepidemiologic studies in situations where there are no adequate comparison groups, developing a LTFU database can be useful in evaluating causation using other methods. Often the evaluation of causality is made based on case reports, using criteria of consistency, statistical strength, specificity of association, as well as the temporal relationship of the association and biologic plausibility. In the case of gene therapy, evidence of vector persistence, vector sequences, and/or vector gene product, especially in the target organ of toxicity, or evidence of immune responses may also support a determination of causality.

7. Oncogenesis and Treatment Induced Cancer

7.1. Molecular Basis of Cancer

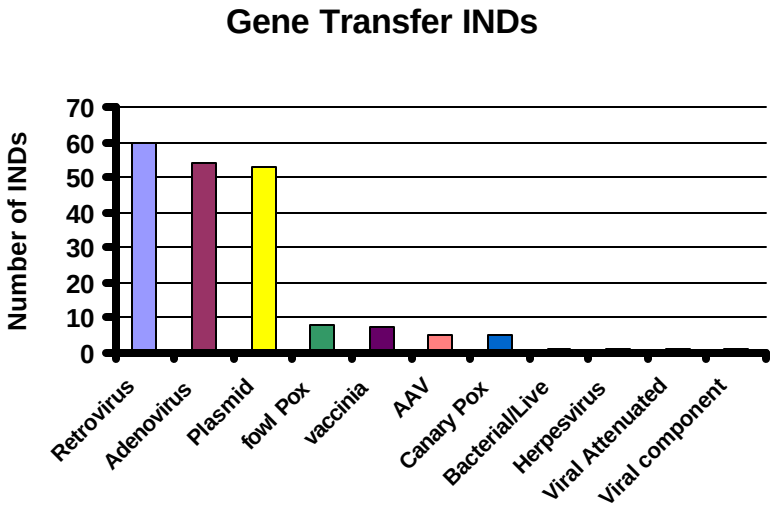
Most, if not all cancers, arise out of an inappropriate growth advantage, caused by the perturbation of signaling pathways or the derangement of a cell's growth cycle regulation. Derangement of the cell cycle machinery is a typical characteristic of most cancers. Environmental, genetic, nutritional, hormonal, and other undetermined factors can contribute to cancer formation.

During the normal life span of a cell, genome integrity is maintained by monitoring or repairing DNA lesions that occur either physiologically (by recombination) or accidentally (through mutations and replication errors). When cells suffer DNA damage, a cell cycle growth arrest at checkpoints in either G1 or G2 permits repair to take place, preventing the accumulation or duplication of mutant sequences. Normal cells experiencing a level of DNA damage that overwhelm their repair capabilities will trigger a self-destruct mechanism (cellular apoptosis), thereby preventing the accumulation of cells harboring mutant genes.

Cancer cells probably acquire a propensity for genomic instability early in the course of tumorigenesis. In due course, a gradual evolution toward oncogenesis is marked by a cell's propensity for replication errors, an impaired ability to repair DNA damage, and the accumulation of multiple genetic mutations that derange the control of cell growth, differentiation, or cell death processes. Along with clonogenic selection, the acquisition of these and other mutations allows newly transformed cancer cells to further develop malignant properties capable of metastasis and resistance to immunosurveillance.

7.2. Viral Oncogenes

DNA and RNA viruses have been studied as important causes of human cancer. The human T-cell leukemia virus type I (HTLV-I) is the causative agent of adult T-cell leukemia (ATL) while the human immunodeficiency virus (HIV), Epstein Barr Virus, human herpes virus-8, human papilloma, and hepatitis C virus (HCV) are strongly associated with several human malignancies (e.g., NHL, Kaposi's sarcoma, Hodgkin's disease, cervical cancer, and hepatocellular carcinoma). DNA and RNA viral vectors are commonly used in gene transfer studies.



The discovery and characterization of oncogenes and the subsequent unraveling of protooncogene functions resulted from the study of retroviruses. It is well established that viral oncogene transduction processes can result in perturbations of normal cellular genes function.

Four mechanisms of viral oncogenesis have been described. The first requires the continuous or recurrent expression viral oncogenes to maintain a transformed phenotype[3]. The second requires an insertional event leading to the expression of an oncogene[4]. The third mechanism of viral oncogenesis relates to the activation of host immune responses triggered by recognition of viral antigens presented on the surface of infected cells (e.g. chronic active hepatitis and cirrhosis)[5]. The fourth mechanism

[3] Example: HPV E2 expression results in up-regulation of E6 and E7 expression. E6 binds indirectly to p53 and ubiquitination and degradation of p53 ensues. E7 binds directly to Rb causing the release of E2F-related transcription factors from Rb. The eventual outcome of E6 and E7 interactions can result in uncontrolled growth and eventual tumorigenesis.

An other example involves altered cellular gene expression and function through viral regulatory proteins(s) that act in trans. The prototype is HTLVI-1. HTLV-1 virus encoded Tax protein interacts with NFκB and other transcription factors to up-regulate transcription of a large number of cell genes that encode cytokines or cytokine receptors (e.g., IL-2, GM-CSF) as well as trans-activating the expression of c-myc, c-fos, AP-1, c-jun that could result in clonal outgrowth and malignant transformation.

[4] Example: transforming retroviruses can incorporate and exert control over cellular growth-related genes or alter cellular gene expression by chance insertion of cis-acting viral regulatory sequences adjacent to these genes (insertional mutagenesis)

[5] The release of inflammatory molecules that recruit other inflammatory cell types, the production of toxic reactive oxygen radicals may trigger proliferative responses by surrounding tissue that may represent an important precondition for carcinogenesis. In this model, the increased proliferation potential of cells increases opportunities for replicative errors that over time can contribute to the loss of normal cell function leading to oncogenesis.

involves a "hit-and-run" event in which cellular transformation is initiated through an initial "hit" that results in the loss of function cellular regulation leading to genomic instability ("run")[6] The hit-and-run concept raises the possibility of an etiological role of viral agents in tumors that lack any viral genes and proteins expression.

Oncogenesis following gene transfer studies is possible and all four mechanisms related to viral oncogenesis could be invoked. Current methods of gene transfer have significant limitations. The inherent integration potential of certain viruses or plasmid sequences, the potential for recombination events, the replication competence of several vector classes, and the possibility a host reservoir and subsequent reactivation (latency) all present major limitations to current gene transfer studies. The lack of control and predictability of where integrating vectors insert within the host DNA can lead to oncogenic protein expression and genomic instability. In vivo administration of viral vectors can trigger a host immune response. Both neutrophilic and lymphocytic inflammatory infiltrates can be seen histologically at sites of injection of adenoviral vectors. Antibody responses to adenoviral vectors have been demonstrated in a variety of animal models, and gene expression with repeated dosing is inversely proportional to the antibody response in some systems. It is postulated that the expression of gene products along in context of viral proteins could trigger humoral and cellular responses that themselves could induce oncogenic transformation.

7.3. Carcinogenicity of Conventional Anti-Cancer Therapy

Increased risks of second cancers have been observed after radiotherapy, chemotherapy, or combined modality treatment, and knowledge gained from studies that explored the relationship of prior anti-cancer therapy to de novo cancer is informative when taken into the context of gene transfer studies. The importance of determining a therapy's potential to induce de novo cancers is exemplified by the following: among 15-year survivors of Hodgkin's disease, second cancer deaths have been reported to be the largest contributor to the substantial excess mortality that these patients experience. Of the many late complications of treatment, second cancers are generally considered to be the most serious.

Estimates of second cancer risk after treatment of various primary malignancies (e.g., Hodgkin's disease, NHL, testicular carcinoma, ovarian cancer, breast cancer, pediatric malignancies) are primarily derived from several sources, including population-based cancer registries, hospital-based cancer registries, or clinical trial series. Of note is that many clinical trials do not routinely collect information on second malignancies, and some do not collect any data beyond 5 years. The lack of long-term follow-up data from clinical trials has been a major impediment in

[6] The expression of Ad5 E1A with either Ad5 E4orf6 or Ad5 E4orf3 can initiate the stable transformation of primary rat cells. Some of these cells may convert to a fully oncogenic phenotype and can form tumors in nude mice. However, when analyzed for the presence of detectable oncogene or viral DNA sequences, transformed cells lacked the continuous expression of viral proteins required to sustain a transformed phenotype. These observations are suggestive of a hit and run mechanism that claims that viral molecules are necessary for the initiation but not the maintenance of cellular transformation (J Virol 75:3089, 2001).

determining the actual incidence of second cancer associated with conventional chemotherapy or radiation therapy.

Commensurate with improvements in life expectancy, several follow-up studies have reported significant increased risks of second cancer following conventional anti-cancer therapy. These studies tend to require the long-term follow up of large numbers of subjects because treatment-related cancers that must be differentiated from an expected background incidence may occur years following initial therapy. For example, the excess risk of leukemia attributable to treatment for Hodgkin's disease peaks 5 to 9 years following initial therapy and levels off after 15 years. The relative risk of lung cancer increases steadily with increasing follow-up time and the risk for breast and thyroid cancer does not become apparent until after 10-15 years of observation.

The carcinogenic potential of ionizing radiation and that of chemotherapy are relevant to the discussion pertaining to the potential carcinogenesis attributable to gene transfer studies. Knowledge gained from pathophysiologic and epidemiologic studies elucidating the carcinogenic effects of anti-cancer therapy are applicable to the perceived risks associated with gene transfer studies. For example, the type of post therapy cellular injury[7], the interaction of therapy with environmental carcinogens and genetics susceptibility[8], and specials risks associated with host factors (immunosuppressed state, subject's age, co-morbid state) are likely to involve similar oncogenic mechanisms discussed previously in this review (e.g.: derangement of the cell cycle machinery, abnormal cellular repair capacity; genomic instability, and increased proliferative potential).

[7] Example: unbalanced chromosome aberrations have been demonstrated with the use of alkylating agent therapy. These aberrations are commonly associated with an inhibition of DNA topoisomerase II and an increased risk of AML.

[8] For example: RB1 is a tumor suppressor gene involved in the regulation of the cell cycle critical to DNA repair. A child with hereditary retinoblastoma who harbor a heterozygous germline mutation in RB1 has a much greater risk of developing osteosarcomas within the radiation field than children who do not have the mutation. Similarly, radiation-associated second malignancies are most common in carriers of the mutated ataxia-telangiectasia (ATM) gene.

8. Hematopoietic Disorders

The hematopoietic progenitor cell (HPC) has two essential properties worth considering when discussing gene transfer studies. First, it represents a self-replicating population of cells that gives rise to HPC descendents. Second, the descendents of HPCs make up the differentiated cells of the blood and bone marrow essential to human life. The HPC can be a primary or an unintended secondary target of gene transfer studies; and therefore, gene therapy could result in the development of life threatening stem cell disorders (e.g. secondary myelodysplastic syndromes or secondary leukemias). Thus, the long-term follow-up of gene transfer study participants to detect hematopoietic adverse events is warranted.

8.1. Insertional Mutagenesis

The process of vector integration into the host genome may lead to insertional mutations with truncation or other deviations from the normal gene product. Insertional events could occur with all known gene transfer vectors. The integration of a viral vector into the genome of HPCs will introduce an unknown factor into the biology of these cells. The well-known effects of chemotherapy on stem cells are seen after an exposure that usually lasts a few months, at the most. In contrast, the viral vectors may be incorporated into the HPCs genome for a much longer period of time, allowing for the possible development of incremental changes that cannot be predicted by current laboratory models. Therefore, long-term complications resulting from insertional events are expected to occur months to years following an initial insult.

An example of retroviral induced insertional mutagenesis leading to a T cell lymphoma in non-human primates as was reported by Donahue, et al (J Exp Med 176:1125, 1992). As a result of a recombination between vector and packaging encoding sequences, a replication-competent retrovirus was produced. These viruses were incubated purified immunoselected CD34+ stem cells from rhesus monkeys and used to reconstitute myeloablated (rhesus) recipients. Six or seven months after transplantation, 3 of 8 stem cell recipient developed a rapidly progressive T cell neoplasm. Analysis of the lymphoma showed that they were clonal, with a common insertion site of the retroviral DNA. The authors concluded that the “data are most consistent with a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis of critical growth control genes, leading to cell transformation and clonal tumor evolution. (Vanin, EF, et al. J Virol 68:4241, 1994).

8.2. Virus Induced Hematologic Syndromes

Clinical syndromes where viruses are known to affect the bone marrow may be relevant to long-term complications following exposure to viral vectors. Although most of the virally induced hematologic syndromes are acute or likely to manifest within weeks of an infection, the discussion bears relevance in the context of viral

vectors with biologic reservoirs and the potential for latency (e.g. replication competence and reactivation in the context of immunosuppression).

Parvovirus is known to induce red cell aplasia. Parvovirus B19 is a single-strand DNA virus that is tropic for human erythroid precursors. Infection results in growth arrest during the S phase of CFU-E's and proerythroblasts. This leads to a cessation of erythropoiesis that lasts until the virus is cleared. This cessation has a minimal clinical effect on health subjects with normal hematopoesis, but in patients with chronic hemolytic anemias, the loss of the ability to produce a reticulocytosis can lead to a potentially life-threatening hypoproliferative anemia. Patients with chronic immunosuppression, most commonly those with HIV disease, may develop a chronic parvovirus infection, resulting in long term red cell aplasia.

Viral hepatitis is known to induce aplastic anemia. Aplastic anemia can occur two to three months after an episode of hepatitis. This syndrome is most common in young males. The disease can be treated with immunosuppression or bone marrow transplantation. The etiologic agent of hepatitis-associated aplastic anemia does not seem to be any of the well-described viruses. (NEJM 336: 1059, 1997)

HIV is a known cause of marrow dysfunction. HIV, a retrovirus is associated with a number of qualitative and quantitative abnormalities of the hematopoietic system. Patients may have isolated or combined cytopenias, or marrow aplasia. HIV infection of the hematopoietic progenitor cell does not seem to be the major factor. It is the infection of auxiliary cells, the macrophages and microvascular endothelial cells that lead to disruption of the supportive matrix of growth factors. (Moses A, et al. Blood 91:1479, 1998)

8.3. Recommendations for Long Term Follow-Up

Because the retroviral vectors can integrate with the nuclear DNA of HPCs, they have the highest risk of producing chromosomal abnormalities over a long period of time. There are no reliable data concerning the duration of this risk, therefore, no upper limit of time for hematologic follow-up can be given at this time. However, based on the experience with alkylator agents, follow-up may be needed for at least ten years and preferable twenty years. For adeno-associated virus vectors, again, no upper limit of time can yet be determined for follow-up. The natural history of the transduction of cells with these vectors has yet to be determined. Thus, data needs to be collected over a prolonged period of time.

9. Neurologic Disorders

The nervous system has a variety of structural and functional features that distinguish it from other body systems. These distinctions have important implications in understanding how CNS injury related to gene transfer products may become apparent.

The nervous system includes both the peripheral and central nervous systems (CNS). The CNS is a highly specialized and structurally distinct organ comprised of the brain and spinal cord. Together, the system exerts important effects throughout the body. Within localized areas of the CNS (e.g., the nuclei), there are a variety of specialized cell types that intermix. Thus, if an insult to the CNS directly or indirectly targets a specific cell type, the sites of injury may be localized within the brain, but not will not necessarily be anatomically uniform.

The normal brain has considerable redundancy in functional capacity resulting in functional plasticity. Hence, for many known neurologic disorders, the extent of cellular damage must be significantly advanced before the disorder becomes clinically evident. An example of disease in which localized areas suffer profound cell loss before clinical manifestation includes Parkinson's disease, where it is estimated that perhaps as many as 80% of dopaminergic cells must be lost before the disorder can be clinically diagnosed and amyotrophic lateral sclerosis where substantial numbers of motor neurons must be lost before clinical signs appear.

Seventy percent of all the human CNS neurons are found in the cortex and 75% of these neurons are located in the association cortex (Nauta and Fiertag, 1986). Nearly all human "higher cortical functions" (e.g., intellect and memory) are carried out by the association cortex. Considering that the neuronal components of the mature CNS do not appreciably regenerate, diseases affecting higher cortical functions become symptomatic or are diagnosed only after large areas, or distinctive specialized areas within the cortex are affected. Therefore for most disorders, early diagnosis is unlikely, even following a careful neurological examination.

Gene transfer strategies likely to represent the greatest insidious risk to the CNS include those strategies that utilize replicating or integrating vectors, vectors with long latency, and vectors whose trans-genes can result in long protracted expression of immunogenic proteins. Chronic exposure to CNS damaging processes could result in neurologic disorders that may not become apparent years following initial administration of an investigational gene transfer product.

9.1. Mechanisms of Nervous System Injury

Several mechanisms of CNS injury have been documented in the literature: 1) inborn genetic defects, 2) direct neuronal and/or glial injury, 3) indirect neuronal and/or glial injury/insults (including metabolic effects), and 4) autoimmune disorders. Gene transfer products could initiate some or perhaps all of these mechanisms of CNS injury. Persistent vectors incorporating into neurons could produce disorders similar

in time course to the genetic disorders (e.g., inadvertent competition for biochemical substrates necessary for normal neuronal function). Stable viral or trans-gene protein expression with inadvertent direct or indirect toxic effects on neurons or glial cells (e.g., TNF, PTH-RP, Ils) could lead to acute or chronic degenerative disorders. Specific vector types could initiates autoimmune injury to the CNS (e.g., autoantibodies, T-cell activation). Lastly, the accidental and unintended introduction of an infectious agent leading to CNS disease (e.g., JC, CJD, other undetected viruses) is possible despite current protective measures. It is likely that host factors (e.g., immunosuppressed host, genetic predisposition to degenerative CNS disorders, children, and the elderly) could influence the long-term incidence of gene transfer adverse events.

Thus, an understanding of the timeframes of development of known CNS disorders regarding the length of time between initiating event and clinical manifestation of a disorder may be informative regarding the potential timeframes between administration of a gene transfer product and appearance of a neurological adverse reaction.

9.2. Clinical Manifestation of Neurologic Disorders

Inborn genetic disorders are obviously present from birth. There are many examples of inborn genetic disorders that do not manifest themselves as full-blown neurological disease until adolescence or adulthood. These include some of the juvenile and adult forms of neuronal ceroid lipofuscinosis (ages of onset 4-10 years and 20-30 years, respectively) and the adult forms of metachromatic leukodystrophy (onset after age 10). Huntington's Disease has onset in the fourth and fifth decades of life with age being inversely correlated with the number of tri-nucleotide repeats present in the defective gene. The hereditary spinocerebellar ataxias have varied ages of onset, but many patients will not come to diagnosis until adolescence and some are not apparent until adult life. Muscular dystrophies, neurologic disorders of muscle, become apparent at different ages for the different forms. Becker's muscular dystrophy and myotonic dystrophy do not become apparent until childhood or even until the person reaches adulthood. Thus, these as well other disorders serve as examples of neurologic manifestations of disease that do not become clinically apparent until after many years of presence of the pathologic process.

Direct infectious disorders of the CNS are also known. Most manifest themselves acutely. However, amongst viral infections HIV and Varicella-zoster are important to bear in mind for the purposes of this discussion. HIV can produce neurologic symptoms that appear to have a substantial lag between systemic infection and development of HIV related dementia. Varicella-zoster is important because it illustrates the capability of a virus to infect neurons and become latent. The infectious agent remains present in the cells and at some time in the future may manifest as an acute disorder. The period of latency may be several decades. Prion diseases, such as Cruetzfeld-Jacob disease (CJD) are also examples of infectious diseases that produce diffuse neurologic manifestations, including dementia and motor impairments, that can be difficult to diagnose, extremely disabling and

ultimately lead to the patient's death (Poser et al., Brain, 1999). In CJD the neurologic symptoms and signs appear to have a long latency period following the initial infection before becoming apparent, often 10 to 20 years post exposure.

Toxic or metabolic insults to specific populations of neurons in the CNS that later lead to manifestation of particular neurologic disorders are also important to consider. For example, Parkinson's disease, a neurodegenerative disease that presents primarily with motor symptoms and signs, becomes manifest often after age 60. While the exact pathologic basis of the disorder is not known, it is believed due to at least several decades of exposure of the dopaminergic neurons to some toxic/metabolic injury. Amyotrophic lateral sclerosis (ALS) is another neurologic disease with primarily motor manifestations that does not become clinically apparent until large numbers of motor neurons have been lost, by which time the affected patient is on a path of steady decline that leads to death. ALS is thought caused by a toxic or metabolic insult to the CNS that is not yet known or understood. The duration of the pathologic process prior to clinical diagnosis is unknown, but the fact there is a typical range of age of development (within the 50s) suggests that there is not a single exposure leading to rapid neuronal death, but rather a long process of injury that results in the clinically apparent disease.

Toxic or metabolic insults to populations of cells in the peripheral nervous system that may later lead to neurologic disorders may also be important to consider. Some selected examples include many of the neuropathies (e.g., diabetic neuropathy, HIV-associated neuropathy, chemotherapy-induced neuropathies) and some of the myopathies (i.e.. the metabolic myopathies). Similar to CNS neuron insults, many of these peripheral nerve disorders do not become manifest for long periods of time following the onset of the metabolic disturbance. For example, diabetic neuropathy often does not become clinically manifest until the patient has had diabetes for 10 to 20 years. Thus, the duration of the pathologic process prior to evidence of clinical neurologic disease may be prolonged.

Autoimmune disorders represent another mechanism leading to neurologic dysfunction. Multiple Sclerosis (MS) and myasthenia gravis (MG) are probably the best-known examples of autoimmune diseases affecting the nervous system. The clinical appearance of MS likely follows some as yet unknown triggering event (speculated by some as an unidentified viral agent) that is thought to occur prior to adolescence. The disorder does not manifest itself generally until the second to fourth decades of life. MG is clearly an acquired immunological disorder in which there are known to be antibodies to the acetylcholine receptor. However, it does not generally manifest until adulthood, with the most common age at onset the second and third decades in women and in the seventh and eighth decades in men. Like MS, the initiating cause remains unknown, and the importance of genetics and triggering events remains unclear.

9.3. Monitoring for Adverse Effects

The relatively inaccessible location of the CNS and the insensitivity of currently available assessment tools make the early detection CNS injury and monitoring of adverse events a clinical challenge. Imaging techniques have made enormous advances over the past 20 years, but their use in the long-term monitoring of gene transfer study participant has significant limitation. The most widely available techniques of computed tomography (CT) and magnetic resonance (MR) imaging can detect focal and widespread disease only after significant CNS injury has occurred. Subtle CNS injury resulting in functional impairment is unlikely to be detected by CT or MRI. For example, in Parkinson's disease, degeneration of dopaminergic cell bodies in the substantia nigra generally cannot be detected because affected cells are indistinguishable from other unaffected cells located within this structure. PET and functional MR (fMR) techniques can assess the functional status of some neuronal systems, but these are limited to a small number of functional systems in the CNS and they can only assess one specific anatomic/functional system at a time. In addition to limited availability, the latter imaging tests are progressively more expensive to conduct, greatly limiting their practical usefulness for ongoing routine long-term safety monitoring of study subjects. Sampling of cerebrospinal fluid (CSF) is invasive, considered a procedure that carries a moderate risk, and often provides little clinically useful information. Brain biopsy may provide sufficient material for pathologic examination and use of molecular studies to assess the effects of gene transfer therapy on the CNS. However, biopsies are invasive, carry a significant risk, and should only be used in restricted circumstances when other less invasive tests are inconclusive or not clinically feasible.

9.4. Summary

The development of degenerative neurologic diseases following participation in gene transfer trials has not been observed to date. However, gene transfer products can theoretically induce CNS pathology via direct and indirect mechanisms.

There is a potential risk of direct infection of the CNS and subsequent acute, subacute, or chronic ongoing injury resultant. Products that can induce long lasting protein production will raise concerns regarding the potential to lead to a slowly advancing injury that could manifest clinical symptoms years after the initial product administration. Vectors and trans-gene products could trigger a nervous system-directed autoimmune disorder that could persist years after the initiating vector has been eliminated.

The nature of the CNS imposes great limitations upon the sensitivity of monitoring techniques intended to detect pathologic processes prior to becoming clinically evident. CNS adverse reactions may go undetected until they become frank clinical disorders. Therefore, if prospectively collected data relevant to potential neurologic complications following gene transfer procedures is desired, subjects should be followed for long periods of time (perhaps 20 years or more) following their participation in gene transfer trials.

10. Autoimmune Disorders

To date, our knowledge of gene therapy and the onset of an autoimmune disease (AID) adverse event is limited. There are aspects of the mechanisms of action of some gene-based therapies that could theoretically result in or contribute to the development of an AID. These concepts will be considered in light of current knowledge that autoimmune responses can be triggered by genetic factors[9], the environment[10], antibody cross reactivity to normal tissue[11], immune complex reactions following the activation of complement[12], and T-cell mediated autoimmune events.[13]

[9] The most common evidence for the existence of a genetic predisposition to AID is in the higher incidence of the disease in monozygotic twins, with a lower but still increased incidence in dizygotic twins and family members when compared with an unrelated population. Most autoimmune diseases are associated with class II MHC, although some are associated with class I MHC. The association between MHC genotype and AID is expected, since autoimmune responses involve T-cells and the ability of T-cells to respond to antigen depends on MHC. Different allelic variants of the MHC may be able to present autoantigenic peptides to autoreactive T-cells. Some MHC genotypes appear to be protective, e.g., HLA-DR2 individuals rarely get insulin-dependent diabetes mellitus

[10] Environmental and other xenobiotic agents can cause autoimmunity. The mechanism of autoimmunity for most agents fall into one of the three following categories: (1) Inhibiting the processes involved in establishing tolerance by deletion. Inhibiting deletion can result in the release of newly generated autoreactive cells into the periphery; (2) Modification of gene expression in the cells participating in the immune response, permitting lymphocytes to respond to signals normally insufficient to initiate a response or allowing the antigen-presenting cells to abnormally stimulate a response; (3) Modification of self-molecules such that they are recognized by the immune system as foreign (Toxicology Letters 2000; 112-113: 421-432). Examples include 1) systemic lupus erythematosus and procainamide use, estrogen replacement therapy, pesticides, and hair dyes; 2) systemic scleroderma and silica.

11 Viruses and bacteria can induce antibody- mediated autoimmune disease via molecular mimicry. In such cases, viral or bacterial antigens are identical or similar to epitopes fortuitously present on a normal cell. Similarly, polyclonal B-cell activation could elicit a humoral response whereby B lymphocytes transformed by certain viruses secrete antibodies against normal cell proteins. Examples include: 1) rheumatic fever following infection with group A strep (inflammation and damage to heart, joints and kidneys), 2) idiopathic thrombocytopenic purpura (ITP) following infectious mononucleosis and other acute viral illnesses and Goodpasture's syndrome following bacterial infection (antibody specific for basement membrane collagen).

[12] The formation of immune complexes can be initiated by exogenous antigens such as bacteria and viruses (or, as in the case of the Arthus reaction, by intradermal administration of large amounts of foreign protein). The mechanism of injury seen in immune complex-mediated disease is the same regardless of which pattern of immune complex deposition is seen (i.e., systemic vs. local).

[13] Dysfunctional T cells can be an effector mechanism of virus-vector induced pathogenesis of AID via down-regulation of T cells that normally suppress immune response to self proteins, i.e., a shift from T_H1/T_H2 cell balance to a predominance of the T_H1 subset. Imbalance of the T_H1/T_H2 T helper cell population is thought to be a general mechanism associated with many autoimmune diseases including multiple sclerosis and Hashimoto's thyroiditis.

10.1. Gene Transfer Studies And Risks Of Autoimmune Disease

10.1.1. RISKS BASED ON VECTOR CHARACTERISTIC

Insertional

Insertional vectors such as retroviral vectors could unmask "AID genes" and express AID in a manner similar to the mechanism proposed for viral activation of oncogenes. However, as in the case of oncogene activation, since AID is polygenic in origin and multifactorial in expression, an insertion initiating AID development might not be detectable until additional insults occurred. Therefore monitoring for this particular type of incident would not appear to be feasible in the clinical trial setting. Other epidemiological approaches (case control studies, etc.) might be of greater value for finding associations such as these.

Replicating

All vectors have the potential to cause AID via formation of cross-reacting antibodies. However, replicating vectors might be expected to have the highest potential for forming such antibodies. The reasoning for this is that the kinetics of viral production from a replicating virus would result in a greater likelihood for mutations and the chance that a new epitope would also be present on cellular protein. The kinetics also argue that replicating viral vectors would cause a greater quantitative antibody response, resulting in higher concentrations of autoantibodies (if formed) and a corresponding greater chance of autoimmune signs and symptoms.

Latent

Similar to replicating vectors, vectors capable of latency (e.g., herpes virus) are also more likely to induce autoimmune processes that may not be detected for long period of time (e.g. years if the target organ for injury is the CNS).

10.1.2. RISK BASED ON DURATION OF GENE VECTOR EXPRESSION

The magnitude of the immune response depends on the dose of immunogen administered. Below a certain threshold dose, most proteins do not elicit any immune response. Above the threshold dose, there is a gradual increase in the response as the dose of antigen is increased, until a broad plateau level is reached, followed by a decline at very high antigen doses. As most infectious agents enter the body in small numbers, immune responses are generally elicited only by pathogens that multiply to a level sufficient to exceed the antigen dose threshold. The broad response optimum allows the system to respond to infectious agents across a wide range of doses. At very high antigen doses the immune response is inhibited, which may be important in maintaining tolerance to abundant self proteins such as plasma proteins.

Considering these factors, the greater the duration of gene vector expression, the greater the potential for a proportional antibody response. The resulting higher

concentrations of autoantibodies might result in a greater chance of autoimmune signs and symptoms.

10.1.3. RISKS BASED ON MODE OF TRANSFECTION (IN VIVO VS EX VIVO)

Because the overall vector dose is expected to be less in gene transfer procedures that involve ex vivo transfer, the risk of AID might be proportionally lessened. However, given eventual entry into the body via cell therapy, the cells, upon destruction, would at that time have the potential for AID risk as outlined above for insertional, replicating and latent viruses, respectively.

10.1.4. RISKS BASED ON MODE OF ADMINISTRATION / TARGETED TISSUE

The route by which antigen is administered also affects both the magnitude and the type of response obtained. Antigens injected subcutaneously generally elicit the strongest responses, whereas antigens injected or transfused directly into the bloodstream tend to induce unresponsiveness or tolerance unless they bind to host cells or contain aggregates that are readily taken up by antigen-presenting cells. Antigens administered solely to the gastrointestinal tract have distinctive effects, frequently eliciting a local antibody response in the intestinal lamina propria, while producing a systemic state of tolerance that manifests as a diminished response to the same antigen if subsequently administered in immunogenic form elsewhere in the body. In contrast, protein antigens that enter the body through the respiratory epithelium tend to elicit allergic responses for reasons that are not clear.

10.2. Patient Specific Factors

10.2.1. IMMUNOCOMPROMISED HOST

Reactivation of latent vectors or increased vector replication is more likely in immunocompromised hosts. Hence, exposure to trans-gene product and/or vector antigens is likely to increase. This is exemplified by the clinical observation that immunosuppressed patients with herpes infections have more active and severe herpes disease. Therefore, it is possible that following greater exposure to immunogenic particles, AID will ensue.

10.2.2. PEDIATRIC POPULATION

Since many autoimmune diseases are not expressed until an older age, it might be expected that the theoretical insults of gene therapy on “autoimmune genes” might be less of a problem than with the adult. On the other hand, theoretically, insertional vectors and latent vectors would have potential to accelerate autoimmune processes such that, in an individual genetically predisposed to AID, the onset of disease could appear sooner than if the patient had developed the disease naturally.

10.3. Long-Term Monitoring

There are limited data available regarding the time it takes to naturally develop an AID. Since there appears to be a genetic basis for the disease, and because the disease tends not to develop until after age 30, it might be hypothesized that that many years (and possibly many environmental insults) are required before an AID manifests clinically. With regards to the discussion above of the theoretical potential of insertional vectors to "unmask" an autoimmune disease, it does not seem likely that this theory could be tested in the clinical trial setting.

Regarding agents that might manifest AID symptomatology through immune mechanisms (e.g., replicating virus vectors and autoantibody formation), some guidance might be obtained from the limited number of studies investigating environmental exposures and the timing of development of clinical autoimmune diseases. Enough information is available to indicate that some environmental agents manifest AID symptomatology within months of exposure. The two best studied autoimmune diseases, discussed earlier, have been SLE and scleroderma. Following acute environmental/occupational exposure, AID symptoms manifested within months. In the case of one study (described earlier) showing an association between AID and silicon breast implants, the manifestations of scleroderma occurred within 4 years of implant in an apparently susceptible group. With regards to xenobiotic agents, the same trend has been observed. For example, the current warnings in the product label for the interferon products (AID risk during treatment) arose from the observation of AID occurring during the 48 week regimen with this immunomodulatory agent. For minocycline, the average time of lupus onset was 19-25 months of continuous exposure, but with a range of 3 days to 6 years.

10.3.1. TYPE OF MONITORING

Given the theoretical concerns and the available evidence of the role of xenobiotic agent potential for causing autoimmune adverse events, gene therapy protocols should monitor for both clinical signs and symptoms of autoimmune disease. In addition, blood samples should be obtained before and after, and possibly during treatment as indicated, to test for the appropriate hematologic markers for determination of autoimmune disease onset.

Short-term

Based on data from the experiences with environmental and xenobiotic agents, a 5 year monitoring period should be sufficient to detect most AID signals as a result of gene therapy in the clinical trial setting. Beyond this period, the background "noise" of naturally occurring AID with confounding variables would make clinical trial assessments of AID of limited value.

Long-term

Case-control studies could be of value in assessing risk of gene therapy beyond the 5 year mark. Educational efforts should be made within the internal medicine community to include obtaining the history of gene therapy exposure for patients with newly diagnosed autoimmune disease. Requiring these to be "reportable disease" could be a method to attempt to follow trends in this area.

10.4. Host Immune Response and Transgene Product

To date, there have been no reports in the literature of an association between any specific transgene product causing notable toxicity. However, there are aspects of some transgene products associated with gene therapy that could be of concern.

10.4.1. Risk of autoantibodies

As in a response to any viral infection, there is the potential for generation of both humoral and cellular immune responses to most gene therapy vectors and most transgene products (Hum Gene Ther 1996; 7(3): 319-31). In addition, transgenes that target certain tissues, if immunogenic, could inadvertently create autoantibodies that are specific for the targeted tissue. For example, gene therapy products specifically targeted towards components of the myelin sheath could theoretically induce untoward immune responses and worsen the very structures that the gene therapy was targeted to improve.

10.4.2. Risk of T-cell mediated autoimmunity

At this time, there are no data available that would indicate that certain transgene products have a greater risk for a dysfunctional immune response than others. However, theoretically, transgenes that might influence the dynamics between the T_H1 and T_H2 T cell subsets could influence propensity towards autoimmune disease. For example, a vector with a transgene coding for IL-2, IFN-γ or TNF-β could be problematic, given the data regarding the role of these cytokines in shifting the T_H1/T_H2 balance in favor of T_H1 cell predominance.

In addition, transgenes might have the ability to influence the mechanism of tolerance established in the immune system, and thus increase the likelihood for development of autoimmune processes. An example might be a transgene coding the production of a member of the APC costimulatory family (B7 family members and cytokines such as IL-1 and IL-6)

10.4.3. Risk of Type I hypersensitivity reaction

If a transgene coded for IL-4 or other cytokines involved with the mechanism of immune system sensitization and IgE production, one could speculate that there might be an increased potential for a Type I hypersensitivity reaction from these transgenes.

10.5. Repeated Administration

10.5.1. Effects of repeated vector administration Humoral

As mentioned previously, the magnitude of the immune response depends on the dose of immunogen administered. Below a certain threshold dose, most proteins do not elicit any immune response. Above the threshold dose, there is a gradual increase in the response as the dose of antigen is increased, until a broad plateau level is reached, followed by a decline at very high antigen doses. As most infectious agents enter the body in small numbers, immune responses are generally elicited only by pathogens that multiply to a level sufficient to exceed the antigen dose threshold. The broad response optimum allows the system to respond to infectious agents across a wide range of doses. At very high antigen doses the immune response is inhibited, which may be important in maintaining tolerance to abundant self proteins such as plasma proteins. In general, secondary and subsequent immune responses occur at lower antigen doses and achieve higher plateau values, which is a sign of immunological memory. However, under some conditions, very low or very high doses of antigen may induce specific unresponsive states, known respectively as acquired low-zone or high-zone tolerance.

T Cell role in hypersensitivity reactions

All normal individuals can make IgE antibody specific for a variety of antigens when antigen is introduced parenterally in the appropriate manner. Several lines of evidence have demonstrated the T_H2 dependency of such IgE responses. The mechanism by which these cells promote B-cell isotype switching appears to involve certain cytokines (e.g., IL-4 and Il-13) produced by these cells. In normal individuals, a balance is maintained between T_H2-derived cytokines that upregulate IgE responses and T_H1-derived cytokines that downregulate IgE responses. Natural events such as viral infection may disturb this balance and stimulate IgE-producing B cells. Therefore, allergic sensitivity may result from failure of a control mechanism leading to overproduction of IL-4 by T_H2 cells and increased IgE production by B cells.

10.5.2. Risks of repeated administration of gene therapy

Humoral response

Considering the factors discussed above, by repeating the dose of gene vector expression, there is a greater potential for a greater antibody response. Should there be any cross-reactivity of any of these antibodies, the resulting higher concentrations of autoantibodies might result in a greater chance of autoimmune signs and symptoms. The timing of the repeat dose would be critical. Closely timed repeat doses could yield the result described above. But if doses are spaced apart in such a manner to enable development of antibodies to the vector, and no autoantibodies are involved, there could actually be a diminished response to the repeat dose of vector.

Cellular response

If the initial dose of vector had resulted in a sensitization immune response, then repeat dosing would increase the potential for a Type 1 hypersensitivity response. Given the association of viral infections in stimulating IgE-producing cells (mentioned above), the potential for this from gene therapy vectors might be increased versus the risk from other xenobiotic agents. An added risk could be posed for a vector with transgene products that are involved with the mechanism of induction of sensitization. For example, if a transgene coded for IL-4, considering the importance of this cytokine in IgE production, there might be an increased potential for a Type I hypersensitivity reaction.

Special consideration of Insertional vectors

As discussed, given the right circumstances, certain vectors have a theoretical risk of inducing AID. Given a genetic basis for some AIDs, and the potential for environmental agents to result in the expression of disease, one could hypothesize that an insertional vectors inadvertent insertion into critical genomic sequences regulating "autoimmune genes" could initiate or potentiate the pathogenic events leading to clinical AID. Repeat dosing of an insertional vector would then multiply the potential for this event.

10.6. Latency/Reactivation

Once they have entered cells, viruses are usually detected by the immune system by directing the synthesis of viral proteins, fragments of which are displayed on the surface MHC molecules of the infected cell. They are subsequently detected by T lymphocytes. To replicate, a virus must make viral proteins, and rapidly replicating viruses that produce acute viral illnesses are therefore readily detected by T cells, which normally control them. Some viruses, however, can enter a state known as latency in which the virus is not being replicated. In the latent state, the virus does not cause disease but because there are no viral peptides to flag its presence, the virus cannot be eliminated. Such latent infections can be reactivated and this results in recurrent illness.

Herpes viruses often enter latency. After an effective immune response controls the epithelial infection, the virus persists in a latent state in the sensory neurons. Factors such as sunlight, bacterial infection, or hormonal changes reactivate the virus, which then travels down the axons of the sensory neuron and re-infects the epithelial tissues. There are two reasons why the sensory neuron remains infected: first, the virus is quiescent in the nerve and therefore few viral proteins are produced, generating few virus-derived peptides to present on MHC class I; and second, neurons carry very low levels of MHC class I molecules, which makes it harder for CD8 T cells to recognize infected neurons and attack them. This low level of MHC class I expression might be beneficial, as it reduces the risk that a neurons, will be attacked inappropriately by CD8 T cells. It also makes neurons unusually vulnerable to persistent infections (White and Fenner, 1994).

10.6.1. RISK OF REACTIVATION

Vectors would probably follow their inherent viral nature. In the case of Herpes-virus based vectors they would be expected to become latent. The vector, like the herpes virus, would probably reactivate under the same conditions as the native virus. Also, the infection by the vector, like the herpes natural infection, would be life-long. Finally, because of their unique ability to target neuronal cells, transgene products will probably be chronically expressed in neuronal tissues.

Herpes-vector-based gene therapy should be thoroughly assessed for neuronal toxicity. Similarly, other latent viral vectors should be monitored for development of tissue/organ toxicity consistent with the location of the latent virus.

10.7. Summary

Gene therapy clinical trial protocols should monitor for both clinical signs and symptoms of autoimmune disease. In addition, blood samples should be obtained before and after, and possibly during treatment as indicated, to test for the appropriate hematologic markers for determination of autoimmune disease onset. In the case of latent virus vectors, added monitoring specific to the tissues/organs within which the vector will be latent should be included as part of safety monitoring

A five-year monitoring period should be sufficient to detect most autoimmune disease signals as a result of gene therapy in the clinical trial setting. Beyond this period, the background “noise” of confounding variables and naturally occurring AID would make clinical trial assessments of AID of limited value.

Educational efforts should be made within the internal medicine community to include obtaining the history of gene therapy exposure for all patients with newly diagnosed autoimmune disease.

Patients who are beyond 5 years of their gene therapy and who have newly diagnosed AID should have their AID designated as a “reportable disease”. Appropriate regulations should be instituted to mandate the collection of these data and the appropriate apparatus should be institute within FDA to collect and analyze these data as gene therapy evolves into routine patient care.