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Editorial

Immuno gene therapy comes into its own

Diseases desperate grown
By desperate appliances are reliev'd
Or not at all
(Hamlet, IV, 3, 9)

The effort to harness advances in basic biology for medical purposes, and more specifically in order to design new forms of treatment, has been a recurrent theme and a natural trend in history. It is hardly surprising that the explosive development of molecular biology has led to the notion that we should try to use genes to treat human diseases.

Biology and technology

In order to tackle severe diabetes it was necessary to know on the one hand that a specific substance produced in the pancreatic islets controls the blood sugar; on the other hand, one needed the technical expertise of protein chemistry to purify insulin. Bringing scientific advances from the test tube to the bedside has always required biology plus technology. Again, gene therapy is no exception: on the one hand we need to understand how gene correction operates in appropriate cells; on the other hand, we need efficient technology for gene transfer. The development of gene therapy takes place at a time when the relationship between research in academic institutions and developments in industry is rather different from what it used to be. Traditionally, new drugs have been discovered by industry and biological processes have been discovered by academic research; but if a 'drug' is a gene that must be packaged in a vector that must be delivered to certain cells manipulated *in vitro* for some time and then introduced into a patient, the separation between the drug and the biological process becomes rather blurred. For these reasons, it has been regarded as axiomatic that marrying academia with industry would benefit gene therapy, although this concept has not been tested, much less proven, in any controlled trial.

Traditional inherited diseases versus cancer

The notion that serious disorders could be the consequence of minute abnormalities in the genome has been innate to classical genetics; yet, it was not a small relief when, first through protein analysis, and then through DNA analysis[1] it was conclusively proven that it was actually true in the prototype case of sickle cell anemia, and in scores of other cases since. If a single base pair change could be the culprit, it became immediately tempting to think that it might not be that difficult to reverse the change: thus, the initial concept of gene therapy was a direct offshoot of understanding the molecular biology of inherited diseases and having available the technology of genetic engineering. At about the same time it became clear that cancer, a condition which had been conveniently used to exemplify to generations of students the dichotomy between inherited and acquired disorders, is itself a genetic disorder of somatic cells: therefore, why not try to cure cancer by correcting its genetic abnormalities, or perhaps by producing locally biological anticancer agents. This issue is not entirely separate from one raised in the previous section, because the treatment of cancer may be perceived by industry as offering a much wider and wealthier market than the treatment of some of the inherited disorders with the highest prevalence in human populations.

Of course the rationale for genetic correction of an inherited disorder *versus* genetic correction of cancer is fundamentally different. In the former case we are often dealing with a recessive condition due to a loss of function; thus, even a very partial correction could have a major clinical impact (for instance, production of factor VIII to yield a plasma level as low as 5% of normal would convert a severe hemophiliac to a mild hemophiliac). In the latter case we are dealing with a condition where one or more somatic mutations have – by definition – produced in somatic cells a phenotype that dominates the scene: therefore, any direct gene correction approach that falls short of correcting 100% of the cells is not likely to have anything but a transient effect. Here we have a good example of how the difference is in the biology, but the technology is paramount, because dramatic improvement in gene transfer efficiency would be needed to make the latter proposition realistic.

For a physician it is a humbling admission to make that at the moment, in the case of solid tumors the hope of cure lies in surgery, not in medicine. Ehrlich's concept of the magic bullet, which worked so well for bacteria, has not worked as well for neoplastic cells, mainly because these are so similar to normal cells. In fact, the history of oncology has abundantly validated a variation on that theme: there is no single 'cure for cancer', but sound work on the mechanism of action of drugs and radiation, intelligent combinations of these agents, and painstaking design and execution of clinical trials can bring about substantial results in many individual types of cancer; rather spectacular, for instance, in the case of certain leukemias, certain lymphomas and germ cell tumors.[2] Once again, it is probably wise to think of cancer gene therapy along the same lines. At the moment, it

seems hard to reverse literally the malignant phenotype: either because we don't know exactly what has gone awry (inadequate biology), or because we cannot do it with enough efficiency (inadequate technology). In the meantime, however, we can apply our ingenuity to using gene transfer in conjunction with other agents and in a variety of ways in order to control a malignant process.

Lateral thinking: the manufacture of safe T lymphocytes and the renaissance of adoptive cell therapies

For over 30 years, the infusion of T lymphocytes into syngeneic or allogeneic recipients has been used by immunologists to probe the function of effector and regulatory lymphocytes *in vivo*.[3] This procedure, originally termed adoptive transfer of immunity to distinguish it from the passive transfer of serum or antibodies, is still used in countless animal models to either induce or treat various diseases, including cancer. Allogeneic bone marrow transplantation (BMT) represents a special setting in which the administration of T cells was at first an inadvertent adoptive transfer associated with the infusion of bone marrow. The donor T cells present in the graft can provide therapeutic benefits, such as antileukemic effects and enhanced donor marrow engraftment, but also life-threatening complications, primarily graft-versus-host disease (GVHD).[4] Of course numerous attempts have been made to separate these two types of effects, but with little success. The only safe and efficient approach developed so far has been to narrow down the specificity of the donor T lymphocytes to a single one, ie, to generate T cell clones[5] (eg anti-CMV). However, once again we have limitations. The main biological limitation is that a narrow repertoire precludes the targeting of multiple antigens: thus, one would miss the antileukemic effect. The technical limitation is the time necessary to generate the clones – racing over a few critical weeks against rapidly progressing disease.

An alternative approach – generating polyclonal T lymphocytes from the donor – is highly attractive, as long as an appropriate safeguard is incorporated. A suicide gene (such as the herpes simplex virus thymidine kinase (HSV*tk*)) that renders the transduced cells specifically sensitive to a prodrug[6–8] would provide such a safeguard; when GVHD develops, administration of the pro-drug should promptly abrogate it. In the original description of this strategy,[6] one of us placed the emphasis on: (1) efficient purification of transduced lymphocytes based on the expression of a marker before their infusion; and (2) faithful co-expression of the marker and of the suicide gene encoded by the vector, in order to ensure that virtually all infused T cells express sufficient levels of the suicide gene *in vivo*.

The group of Claudio Bordignon in Milan has now reported on the use of precisely this strategy in eight patients. Five patients did not develop GVHD; the three who did were treated with ganciclovir, which reversed signs of GVHD in two of them.[9] It is impossible to extrapolate from such small numbers, but this pilot study is important for at least three reasons. (1) From the evidence of two patients, it appears that the strategy works; this is good news for a field that is in dire need of real clinical results. (2) Not all cells always commit suicide: this is probably because marker-negative cells (as well as 'pseudo-transduced' lymphocytes[10]) are simply not eliminated. (3) An incomplete response to ganciclovir could result from the HSV*tk* expression being insufficient, or only transient, or both. Dual-promoter vectors are often subject to transcriptional interference.[11,12] This could lead to dissociation between expression of the marker gene (under the transcriptional control of the 5′ long terminal repeat), and the suicide gene (under the control of a downstream internal promoter[9]), despite the integration of an intact copy of the vector. Thus, lymphocytes cannot be made safe by just achieving a high level of purification; in fact, it would be misleading to rely only on purification until co-expression is rigorously established and maintained. One of us has recently demonstrated, also in human T lymphocytes, that the problem of transcriptional interference and unreliable gene co-expression can be solved with optimized single promoter dicistronic vectors.[13] Fortunately, we know from dose–response studies in allogeneic BMT recipients that small doses of donor T cells are not sufficient to cause GVHD,[14] suggesting that there may be a tolerable low level of infusion of nontransduced T lymphocytes.

Policies, politics and publicity

Gene therapy was regarded with great respect throughout the 1980s, and hardly any article recording the cloning of a disease-related gene failed to conclude that genetic correction was now in the offing. A sense of urgency in producing therapeutic applications was created not only by venture capitalists who were investing in biotechnology companies; but also, quite independently, by another group animated by a far more personal, pressing and understandable vested interest, namely the patients themselves. Today patients may feel encouraged by hearing that there are some 140 gene therapy protocols active in the USA alone. However, it is not equally widely publicized that (apart from some marking studies of great biological interest but not having therapeutic intents) the large majority of these are 'phase I' studies, which means that they are only meant to test the new treatment for its potential side-effects, not for its therapeutic efficacy. At the time of writing, we cannot boast of gene therapy having unquestionably conquered any disease. Does this mean that we miscalculated, or that we are too slow, or simply that we are too impatient (after all, it took some decades from the finding that pancreatectomized dogs develop diabetes to the time when the first diabetic patient was able to buy insulin in the pharmacy)? In 1995, Arno Motulsky and Stuart Orkin were asked by the US National Institutes of Health to assess the status of gene therapy. The resulting report was a critical but balanced appraisal of what is real and of what is realistic, a model of objectivity, and a good shortlist of commonsense recommendations. By the front-line workers the report was read as a warning that 'rush to press' and 'rush to the clinic' was not a good idea in the area of gene therapy any more than in most other scientific endeavours; and therefore serious scientists and serious clinicians welcomed the report. Unfortunately, for those that like simple dichotomies, the report could also be read as heralding an era of gene therapy scepticism, following the era of gene therapy enthusiasm. Another factor in the backlash has probably been a flurry of premature claims and premature hopes fed by hypes, which is not just the media's, but our responsibility to curb. This is another reason why the article with real results by Bordignon's group is so important.

Biology and bio-ethics

Since the article[9] reports what is at the same time an experiment and a clinical study, questions must be raised on both the scientific and the clinical side. At the scientific level, an ideal resolution to the problem of co-expression might be the production of a chimeric protein obtained by fusing the marker gene to HSV*tk* (provided it will not prove immunogenic). In addition, it is not clear for how long expression of HSV*tk* will continue. There are numerous precedents in other cell types where retroviral expression is sustained *in vitro* but not *in vivo*.[15] For this reason, *in vivo* studies remain a crucial test of this therapeutic strategy. Recent studies have in fact shown that HSV*tk*–transgenic donor T cells capable of inducing GVHD in recipient mice were effectively eliminated by the administration of ganciclovir.[16] However, experimental proof that virally transduced (as opposed to transgenic) lymphocytes can be eliminated in the same way is still lacking, since no such studies have been conducted to date in an animal model, or in mouse–human xenochimeras. Therefore, one must question whether it was appropriate to do the test directly at the clinical level. Fortunately it appears that the experiment has worked neatly in two patients, although in the third patient insufficient expression of HSV*tk* may have accounted for the failure to eradicate GVHD.

These recent studies attest to the new prospects for adoptive cell therapies enhanced by genetic modifications of the infused lymphocytes. Future pre-clinical and clinical tests of our ability to eliminate these cells reliably will be crucial. Once the safety of this strategy is thus fully validated, it will provide the go-ahead signal for other modifications of T cells that may render them more effective therapeutic agents, opening up the prospect for novel therapies beyond the realm of transplantation.

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