

“Genetic Doping” with erythropoietin cDNA in primate muscle is detectable

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Forthcoming “genetic doping” is predicted to be undetectable. In the case of recombinant human erythropoietin (rhEPO), a hormone used in endurance sports, it is being predicted that exogenous drug injections will be replaced by the transfer of the corresponding gene into some of the athlete’s own cells. The hormone thus produced inside the organism is assumed to be completely identical to the physiological one. Our results show that this is not the case and open up optimistic prospects for antidoping control involving gene transfer.

Doping in sport, with very few exceptions, arises from misused medical treatments. This is the case for rhEPO, a hormone that stimulates red blood cell production and that has become a key element of doping in endurance sports. Treatment with rhEPO currently requires repeated injections of recombinant hormones obtained from nonhuman cells, i.e., Chinese hamster ovary (CHO) and baby hamster kidney (BHK) cells, into which the human gene of the hormone has been inserted. Natural endogenous and rhEPO were shown to present different isoelectric profiles, probably the result of altered posttranslational modifications that are species- and tissue type-dependent. This difference has allowed for the development of a test to detect the presence of rhEPO in urine, a test that is currently used in antidoping controls [1].

Genetic technologies are expected to change the very nature of medical treatments. For instance, it is now conceivable that administration of an exogenous therapeutic protein will be replaced by introducing the corresponding gene into some of the patient’s own cells. It is almost inevitable that athletes will exploit such medical progress in an effort to elude detection by sport authorities charged with curbing doping practices. Doping practices, in addition to being the focus of regulatory issues, may also severally and

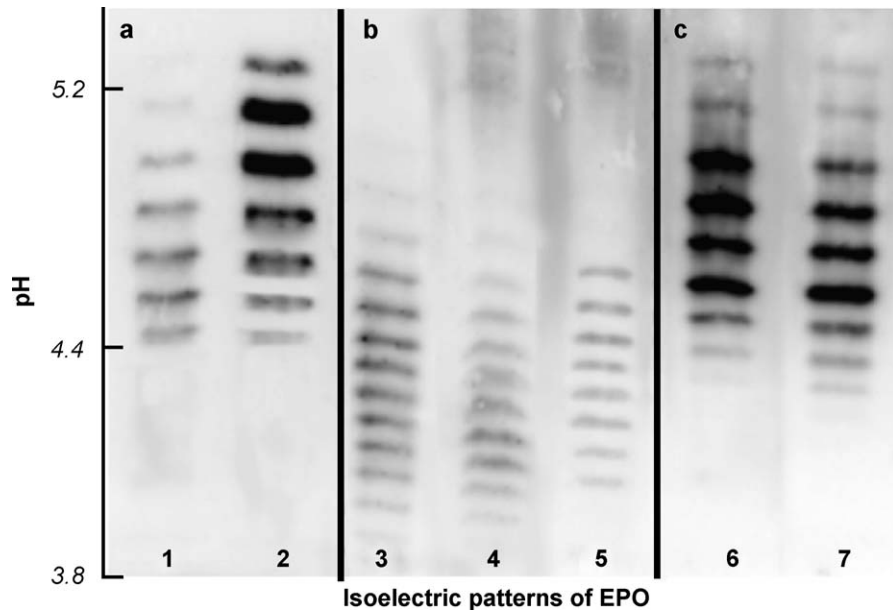
adversely affect the health of athletes that engage in such practices. Doping by gene transfer may compound these adverse side effects because of direct toxic effects, persistent gene expression, or potential insertional mutagenesis [2,3]. Furthermore, the assumption that these new methods of doping will yield proteins that are identical to the endogenous gene product, thus making detection impossible, may not be the case.

To compare the isoelectric profiles of physiological EPO and hormone resulting from *in vivo* gene transfer, we have adapted for serum analysis a method previously developed for urine [4]. Using this method, samples from cynomolgus macaques were analyzed for the serum recombinant EPO profile before and after transfer of the homologous cDNA into skeletal muscle by injection of recombinant adeno-associated virus [5]. Transgene expression was controlled by a doxycycline-regulatable system [6].

The physiological isoforms of the simian hormone were very similar to those of human urine EPO (Fig. 1b). Induction of transgene expression in these macaques resulted in overexpression of a hormone presenting a pattern strikingly different from that of the endogenous isoforms (Fig. 1c). The transgene-derived isoforms resolved with isoelectric focusing at higher pH, a finding more characteristic of recombinant EPO than endogenous EPO (Fig. 1a). In primates, EPO is primarily synthesized by renal peritubular fibroblasts [7]. The distinctive isoelectric pattern of recombinant EPO produced by skeletal muscle emphasizes the importance of cell type on the characteristics of recombinant EPO.

It is noteworthy that the structural features responsible for the described differences between the isoelectric patterns of physiological human urinary EPO and those of recombinant hormone are not yet clarified [4]. The newly observed differences in the macaque serum

Fig. 1. Isoelectric patterns of erythropoietin. (a) rhEPO from CHO cells (lane 1) and BHK cells (lane 2). (b) Physiological EPO from human urine (lane 3) and macaque serum (lanes 4 and 5). (c) EPO from macaque serum after gene transfer in skeletal muscle (lanes 6 and 7). Serum samples (5) and (6) are from the same animal before and after gene transfer, respectively. Specific detection of EPO was obtained by double-blotting following isoelectric focusing. Cathode is at the top.



between the pattern of physiological EPO and that from transduced muscle are every bit as striking and require further study. Because a previous report [8] indicated that EPO extracted from serum was not as different in isoform distribution from recombinant EPO as was urinary EPO, the difference that we report here between the endogenous and the transgene-derived product from the serum samples is even more relevant. However, because the current test for rhEPO in sport uses urine, our study will have to be extended to this biological fluid.

The biological effects of recombinant EPO from genetically engineered muscle have been demonstrated in animal models [9,10]. However, our observations indicate that this recombinant EPO, like the other sources of rhEPO, is not identical to the physiological hormone. Skeletal muscle, since it is an easily accessible and efficiently transduced tissue, is likely to be the target tissue of choice for genetic doping. Although other methods of gene transfer exist and may be exploited for gene doping, and such methods are yet to be investigated, our results provide encouraging evidence that doping by gene transfer will likely not go undetected at least when skeletal muscle is the target.

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