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Title:

Pre-clinical Safety Testing for Biotechnology-derived Pharmaceuticals

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Introduction

The aims of this presentation are, first, to summarize the characteristics of biotechnology-derived pharmaceuticals, second, to figure out their possible inherent problems using the LIF (leukemia inhibitory factor) as an experimental sample cytokine, which hopefully explain a mechanism of sample hazards, and to understand the future strategy for the safety-study, and third, to propose future safety prospective, because there's sometimes no regular animal experiment is available to guarantee human safety other than experiment with a humanized animals with transgenics and/or knock out mouse.

1. Increasing number of biotech-products, and their characteristics.

Contrary to the conventional ordinary drugs shown on the above panel (Figure 1), the function of the biotech-products shown on the lower panel is known from the very early stage of drug-development in general, before reaching to the final products, although the effects on the experimental animal may be different from those in human. Other aspect give rise to a unique sight of characteristics of the biotech-products which seem to have been transient from the conventional drugs toward the future peptide product (Figure 2). One should learn about the details in how to manage of receptor-ligand system as well as of the signal transduction mechanism

during the era of biotech-pharmaceuticals for the future medicine's research toward the peptide products.

Biotech-pharmaceuticals are, however, not only a future drugs, but presently avaialble, rapidly growing subjects. Number of biotech-pharmaceuticals approved in Japan for marketing increases steadily over the last fifteen years (Figure 3). Figure 3 shows those accumulated number of biotech products on the ordinate along with the year on the horizontal. Even if, not all are available for marketing at this moment, a percent-share of biotechnology-derived pharmaceutical items approved in each year is now reaching over 10%.

2. Receptor oriented "unique" toxicity

Let's see a little more about the characteristics of actions in biotechnology-derived pharmaceuticals. Action of biotech-products are induced essentially through a binding of ligands to relevant receptors. Since the action of biotech-products is often pleiotropic, one should carefully examine what might happen, i.e., whether or not accidental expression of receptors might be induced in other non-target organs. Most of cytokines and other bioactive materials have an expression of their receptors in more than one tissue/organ. Their function in one organ is sometimes completely different from the action in the other organ, in terms of their phenotypes in cellular function, which is sometimes undesirable for human-use. Such

receptor-specific actions in addition to pleiotropism include species specificity, redundancy, subunits-oriented dose response, and possible death signals. Basic strategy of animal selection for safety evaluation will be presented. (Figure 5)

3. Use of transgenic (Tg) animals to observe a receptor-mediated relevant reaction

Since an essential function of biotech-products are of receptor- mediated, no reaction is supposed to be taken place when receptors were not available in the testing animals; namely, no receptor induces no reaction. Thus, relevant animals are required to develop to see a relevant biological reaction. Such an in vivo-testing system is really needed because some cytokines, for example, are induced through an induction of secondary gene-expression, in vivo.

Humanized mouse production program at our laboratory was started due to above mentioned background. High affinity IL-3 receptors (IL-3R) are composed of αβ-heterodimers. In order to analyze the functions of those human factors onto relevant human receptors expressed in animals, we have established Tg mice that express the α chain and the β chain of the IL-3 receptors, respectively. An MHC-Ld or a human β -actin promoter was used for ubiquitous expression. These Tg mice with artificially over-expressed IL-3 receptor-subunits provided a good tool to resolve a question in function of the hetero-dimerization and the receptor-sharing in the cytokine network.

Unexpected over-expression of those receptor genes were observed in a variety of tissues, and unexpected pleiotropic actions when treated with human IL-3 were efficiently predicted in these receptor Tg animals.

4. Artificial expression of leukemia inhibitory factor

Leukemia inhibitory factor to be present is another typical sample which has widely distributed pleiotropic action; namely, M1 cell differentiation, DA-1a myeloid cell differentiation, myoblast proliferation, proliferation of megakaryocytic progenitor cells, neuronal cell differentiation, and further supporting that embryonic stem cells do not differentiate but rather proliferate as a self-renewal. (See Hilton's review in Figure 4). Effect on embryonic stem cells is critical, because maintaining of pluripotencies is know to be supported by LIF. When one withdraws the LIF, embryonic stem cells immediately start to differentiate, and go into random organogenesis. In vivo, on the other hand, a constitutive expression of LIF is a rule during the developmental stage, especially during blastcyst-stage. These evidences lead to an idea that an expression level of LIF can not be stable during the development, and arises a question as to whether or not the expression of LIF changes during the embryonic development?!

Construction of two different expression vectors, driven by MESV long terminal repeats and the PGK for an LIF-cDNA were established. Several ES clones with different LIF expression were obtained, i.e. two with high

expression from both vectors, one with middle expression from MESV clone, and two mild expressors from each vector. When one tried them to produce chimeric mice, none of high or middle expression clones provided any pups, only mild expression clones provided pups, thus suggesting no vector-oriented specific differences.

Accordingly, requirement in expression of the LIF is relatively limited in stage during the development, and further, an evaluation of the relevancy must be extended even into an expression-stage specificity. Unfortunately, regulation of the expression is not freely possible yet, specifically site specific expression and/or stage specific expression are very limited. Although it may be a dream at this moment, however, such regulation for site-specific and/or stage specific expressions are essential technique to develop future biotech-products for the next century.

5. New experimental animal development

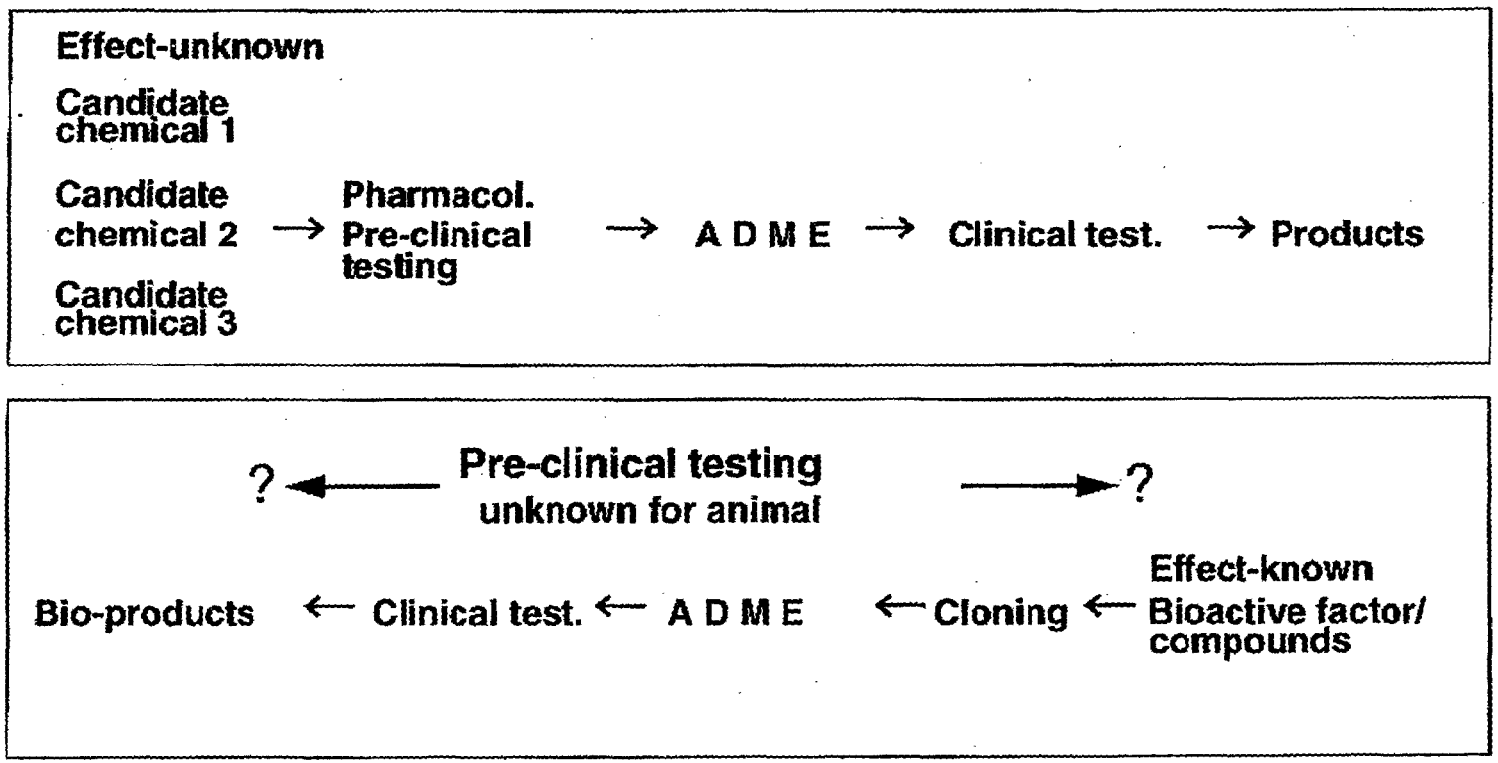
What we should do today, on the other hand, is not simple but limited. Strategy of animal selection shown in Figure 5 is based on a relevancy of animals to testing materials. For these purposes, in vitro studies would be the first choice to define such relevant species. To define it by counterpart messenger RNA expression would be the most help. Including a possible case where one could not find a relevant species, Tg mice carrying the relevant human receptor may provide a useful information, though not fully

informative, but limited.

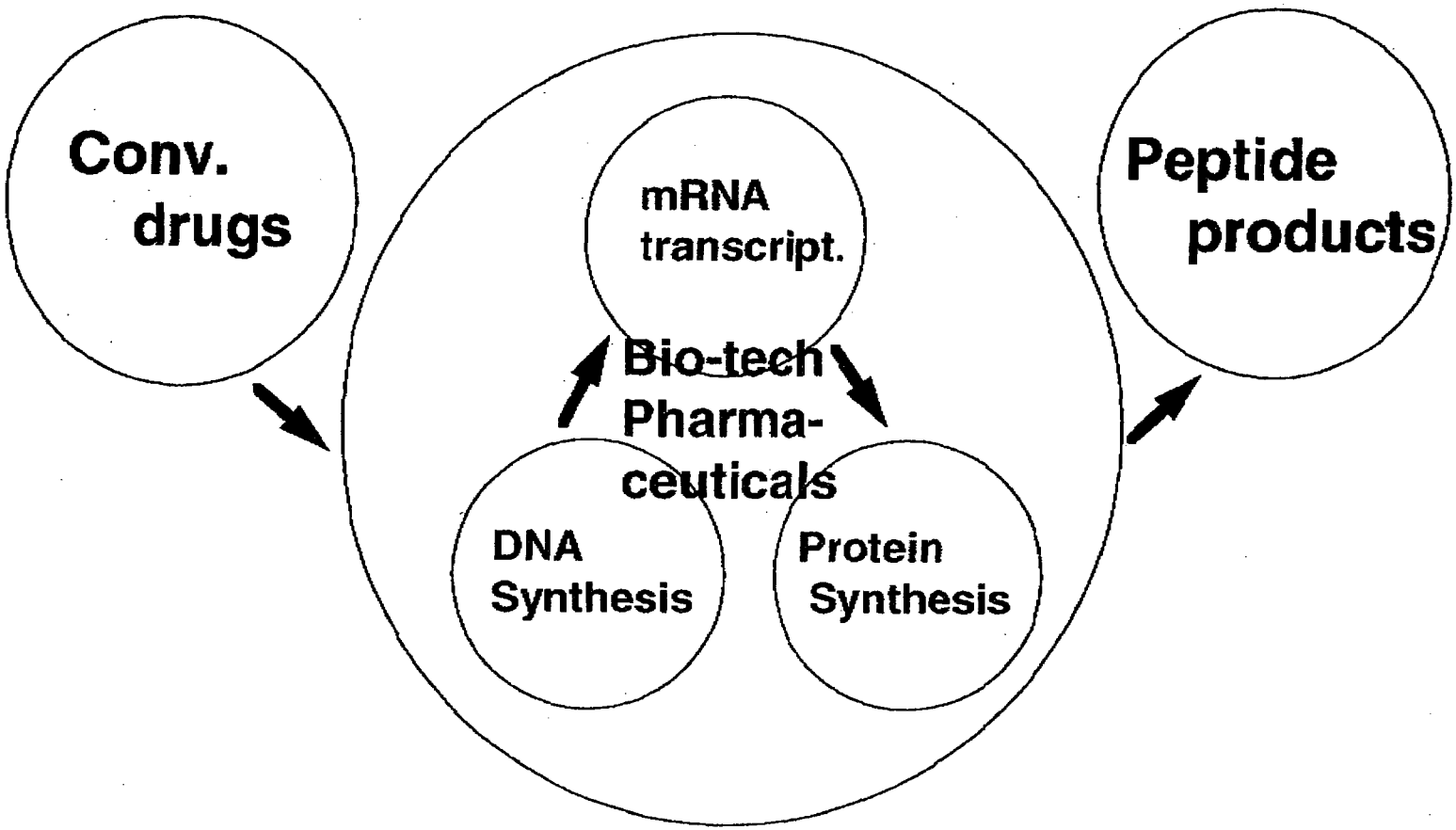
Regular animal study is only applicable when the material has a cross reactivity to testing animals. From the other aspects, murine homologous gene products may be informative for surveying their pleiotropism, but questions for carcinogenicity and/or immune toxicology would never been satisfied even by this system, and again this should requires another type of new testing methods. For those purposes; the former case may be satisfied only by a production of Tg mice carrying receptors for human type ligands, and the latter case may not be fully evaluated by the presently available science, because it requires to develop a mouse replaced their MHC with human counter parts. Namely, in such cases, people should develop murine MHC-K.O. and human MHC kick-in mouse, i.e. humanized mice. None profit organization for an international animal resource to develop such bio-tech recombinant animals for drug-safety is needed to be developed and established. Development of biotech-products are now requests a promotion of rapid progress of science for future toxicological safety evaluation.

Presentation will be based on a document presented by the author at the Fourth International Conference on Harmonisation, held in Brussels on 16-18 July, 1997.

Chemical drug vs. Bio-pharmaceutical

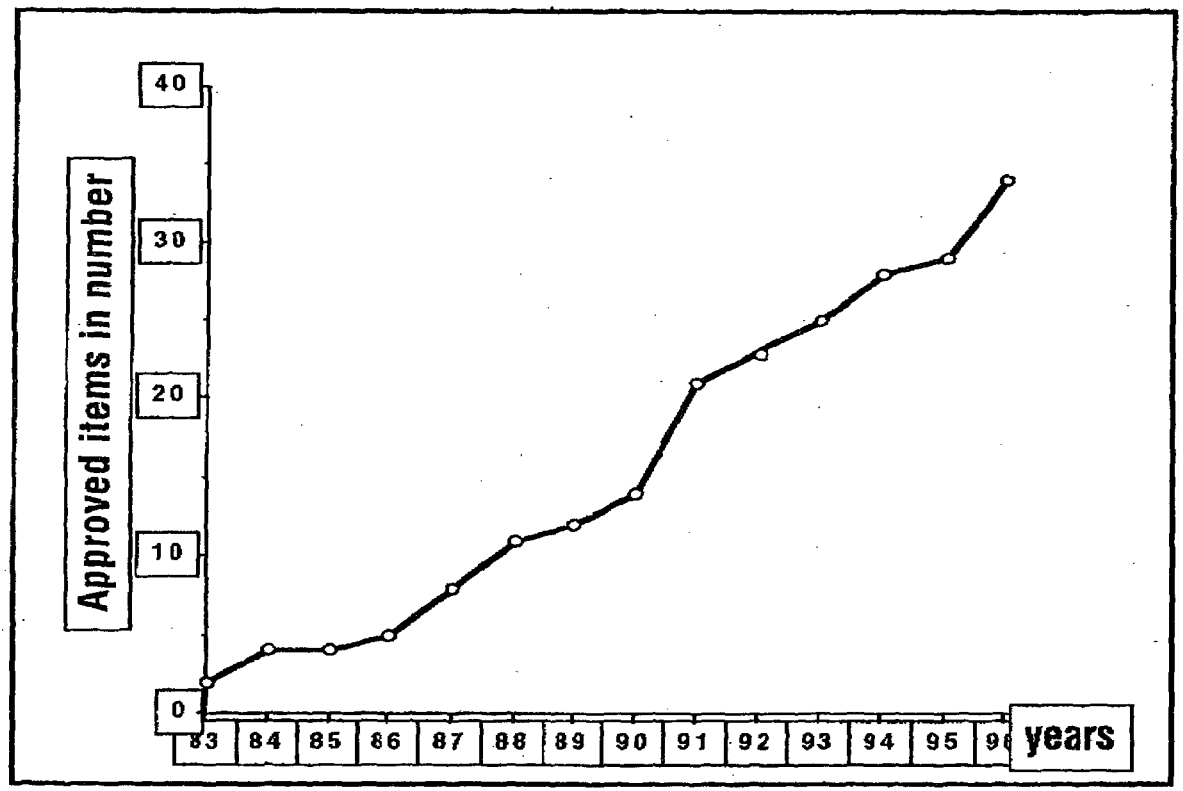


Biotechnology-derived pharmaceuticals
-their transient nature-

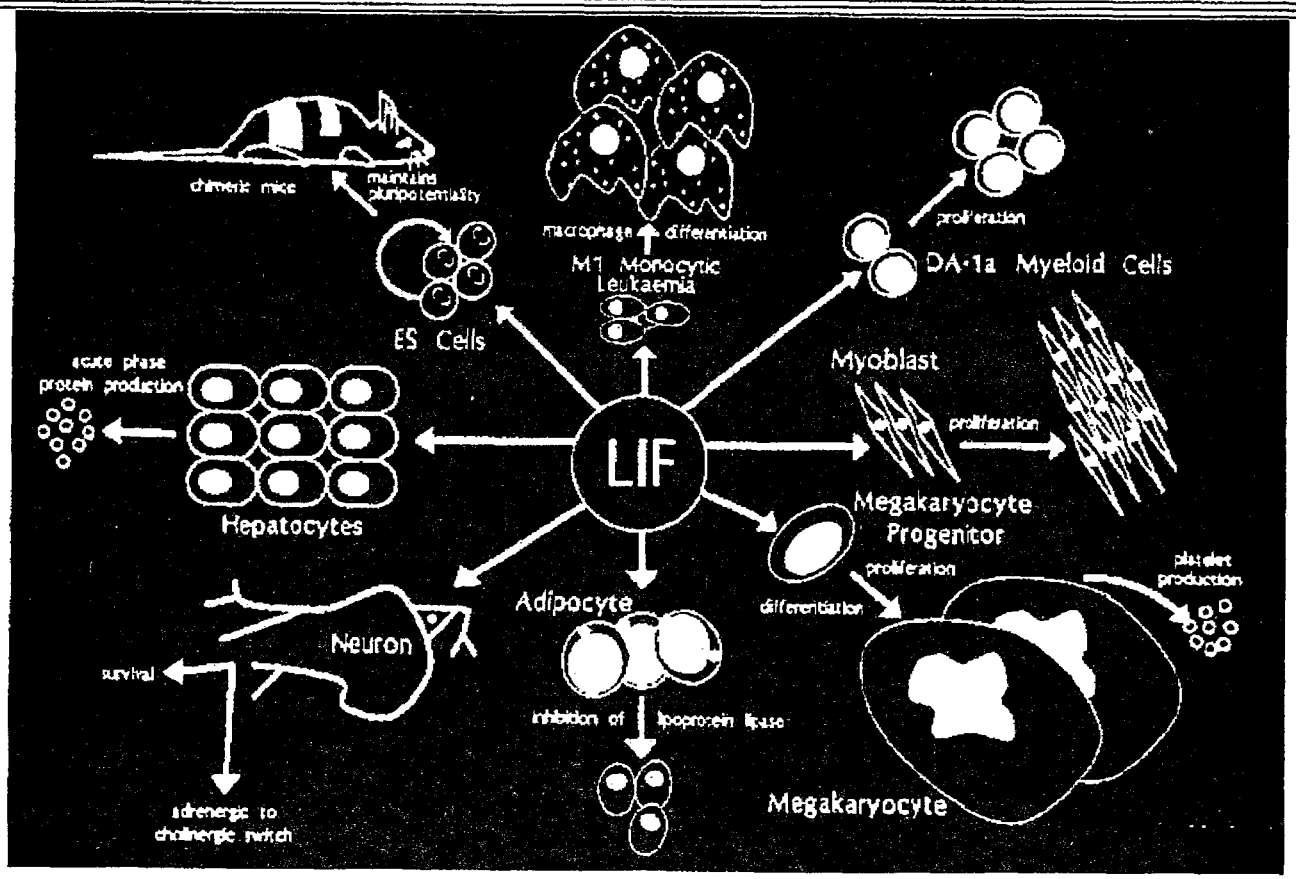


Biotech-Product Increases in Number

Cumulative number of bio-pharmaceuticals approved in Japan for marketing since 1983



Leukemia Inhibitory Factor



D.J.Hilton, TIBS, 1992

Species Selection For Testing Biotechnology Products