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Editorial

Safety for vaccine(e)s

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The terrible doubt associated with the use of a polio vaccine in the U.K. in the last years (eventual transmission of the agent provoking the new form of Creutzfeldt Jakob Syndrom) shows again the urgent need for the use of safer (with respect to biological safety) production methods for vaccines (and biologicals, in general) which are applied to the whole population. In a general accord, safety issues are of utmost importance, because vaccination should lead to the prevention of diseases or epidemies, but should in no case lead to other health problems or life threatening diseases.

Today's viral vaccines, which are used in the developed countries, are generally produced in animal cell cultures, indicating that in addition to the viral inoculum and the downstream processing, the production cultures, the culture conditions, the production media, etc. are major issues of safety considerations. In the following, these issues will be described briefly with respect to the cell substrate, the media issues, the detachment agents and the virus inoculum whereby the emphasis is put on the presence/absence of animal derived substances during the production process:

1. From the point of view of the choice of cell substrate, the vaccine producers and the regulatory agencies always took a rather conservative, protective attitude, and continuous tumorigenic cell lines have never been accepted for the production of dead, inactivated or live attenuated viral vaccines. This is still actual today, although experts, like Petricciani (1993) or Meslin (1994), proposed the use of continuous tumorigenic cell lines, like BHK21 C13, for the production of viral vaccines (e.g. rabies), in particular, for the developing countries, because vaccines produced with such cell lines would be much cheaper and much easier to produce in large scale suspension processes (e.g. production of foot and mouth disease virus (FMDV) (Radlett et al., 1985) and rabies virus (Pay et al., 1985) under serum-containing conditions for veterinary applications; production of an experimental rabies virus vaccine under serum-free conditions: Merten et al., 1994; Perrin et al., 1995). The move to continuous tumorigenic cell lines is principally acceptable because modern molecular biology based methods allow for the detection of sequences of oncogens eventually present or an assessment of the quantity and the state of residual cellular DNA in the final vaccine (Morgeaux et al., 1993; Petricciani, 1993).

   Despite or probably due to this conservative and protective attitude of the regulatory agencies, primary cells are still used as substrate for vaccine production and such practice should be stopped as soon as possible in order to avoid the introduction of adventitious agents derived from the sources of these primary cells presenting a potential threat to the vaccinees.
2. The second important point of safety concerns deals with the composition of the culture medium. Due to historical reasons, but often also due to convenience and financial reasons, all classical vaccine production processes make use of animal derived substances. However, the use of serum is associated with several disadvantages, inter alia, the potential introduction of contaminations and adventitious agents (viruses, prions, . . .) into the final product.

   In a first line, serum-containing media are generally used for the biomasse production phase (cell growth phase before virus inoculation) and sometimes also for the virus production phase (period after virus inoculation up to the harvest of the virus suspension).

With respect to the use of serum-containing medium, it can be stated that the use of serum-free/protein-free media is possible for the production of vaccines. In the case of the use of primary and established diploid cells, the use of serum-free media is not straightforward because only rather complexe serum-free media supplemented with one or several growth factors (Maurer, 1986) can be used and because the development of such media for the large scale applications is difficult and has not been reported up to date. However, in the case of continuous non-tumorigenic and tumorigenic cell lines, such as Vero or BHK21 C13 cells, respectively, the situation is quite different. Such cells can easily be adapted to growth in serum- and even protein-free media and such cultures are capable to produce viruses as efficiently as or even better than comparable cultures in serum-containing medium: Rabies virus on BHK-21 cells (Merten et al., 1994, 1999; Perrin et al., 1995) and on Vero cells (Merten et al., 1994), or polio virus on Vero cells (Cinatl et al., 1993; Merten et al., 1994) for small scale cultures; Merten et al. (1997) for reactor cultures; Merten et al. (1999) in a protein-free medium free of animal derived substances. Although Vero cells are useful for the production of high virus titers (e.g. production of polio virus (Beale, 1981) or production of rabies virus (Petricciani, 1987) and a Vero based killed polio vaccine was licensed in France in 1982 (Montagnon et al., 1984), efforts for the development of serum-free media for vaccine production were rather limited/timid from the side of the industry. The only recently developed vaccine based on a totally serum-free production process is an influenza vaccine produced on MDCK cells (Brands et al., 1999).

3. In a further instance, trypsin is a necessary substance for passaging of all cell substrates used for vaccine production and presents a certain threat due to its origine from animal pancreas (mainly porcine derived). In the case of obligatory adherent cells which are growing in serum containing as well as in protein-free media in adherence (e.g. Vero or MDCK cells), passaging is based on the use of detachment methods. Trypsin, the main detaching agent, can easily be replaced by non-animal derived proteases (e.g. Merten, 1999) or by recombinant trypsin produced in microorganisms (Hohenblum et al., 2000).
4. The further processing of viral vaccines makes equally use of animal derived materials. Here only the addition of stabilizing agents during processing and particularly in freeze drying formulation should be mentioned (for instance, serum albumin might be replaced by recombinant human serum albumin (e.g. New Century Pharmaceuticals, Inc.). In any case, this issue is of equal safety concern as those issues concerning the use of animal derived materials for all other steps in the vaccine production.
5. The viral inoculum is itself produced in animal cell culture, and with respect to the preparation of this inoculum (cell culture, virus inoculation, medium preparation, etc.) the same safety considerations are applicable as for the viral vaccine finally produced. lt is evident that further issues like passage number or viral titer, etc. which are not directly related to the cell culture conditions, are of equal importance.

Thus it becomes evident that all issues concerning the biological safety with respect to, for instance, the cell substrate, the medium choice and the detachment method used can relatively easily be solved and that today relatively safe and economically viable alternatives are available for the production of most of the important vaccines for human use (e.g. production of polio virus by Vero cells in serum-free medium). Therefore, the actually most important consideration – the economical consideration – of vaccine production should be replaced by the second most important one which concerns the biological safety issues, in order to develop, validate and approve new production methods for biologically safer vaccines.

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