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INTRODUCTION

In 1975, forty-five people severely bitten by rabid dogs and wolves in Iran were treated after exposure with a new rabies vaccine produced in cultures of human diploid cells. All except one also received one injection of rabies immune serum. This treatment, in contrast to past experience with other vaccines, resulted in protection of all individuals against rabies.\(^1\) This resounding success has been repeated in trials in Germany and the U.S.A. using 5 or 6 doses of human diploid-cell strain (HDCS) rabies vaccine and human rabies immune globulin.\(^2,3\) Thus, almost a century after the postexposure treatment of man began, effective antirabies prophylaxis appears to have been achieved.

With few exceptions, rabies is a problem of impoverished areas of the world, where the annual per-caput expenditure on health care is often far less than the cost of a single ml dose of HDCS vaccine. As a consequence, potent tissue-culture vaccine is seldom used in the Third World. The need for an effective but less expensive method of treatment prompted us to investigate the possibility of administering potent vaccine more economically and efficiently than at present. Our previous studies have shown that substantial titres of antibody can be achieved with small quantities of HDCS vaccine administered by the intradermal (i.d.) route and that the cost of vaccination can be reduced considerably.\(^4,5\) In this paper we report the antibody and cell-mediated immune response of man to multisite i.d. vaccination and application of the method to postexposure protection of rabbits. It appears possible that the i.d. route could be applied successfully to the postexposure treatment of man.

MATERIALS AND METHODS

The Studies

Approval for the volunteer study was given by Northwick Park Hospital ethical committee. HDCS vaccine (0-8 ml) was given i.d., on a single occasion, to ten volunteers at 8 sites on the medial and lateral aspects of the upper arms and thighs. Blood samples for tests of lymphocyte transformation and antibody determination were taken before vaccination and 10, 14, 21, and 42 days later. A further blood sample for antibody titration was taken on day 100. Four

31. Cavalli F, Sonntag RW, Jungi F, Senn HJ, Brunner KW. VP 16-213 monotherapy for remission induction of small cell lung cancer, a randomized trial using three dosage schedules. Cancer Treat Rep 1978; 62: 473.

32. Egan RT, Carr DT, Prysock S, Rabin J, Lee RE. VP 16-213 versus polychemotherapy in patients with advanced small cell lung cancer. Cancer Treat Rep 1978; 62: 949.

33. Tucker RD, Ferguson A, Van Wyk G, Steyl R, Hewston R, Levin W, Rad RF. Chemotherapy of small cell carcinoma of the lung with VP-16. Cancer 1978; 41: 1710.

34. Cohen MH, Broder LE, Posewicz BE, Ihde DC, Minna JD. Phase II clinical trial of weekly administration of VP 16-213 in small cell bronchogenic carcinoma. Cancer Treat Rep 1977; 61: 489.

35. Hansen M, Hirsch F, Dombrowsky P, Hansen HH. Treatment of small cell anaplastic carcinoma of the lung with the oral solution of VP-16-213 (NSC 141540): 4'-demethylepipodophyllotoxin 9-(4,6-0-ethyldene-β-D-glucopyranoside). Cancer 1977; 40: 633.

36. Egan RT, Ingle JN, Creagan ET, Prysock S, Kivos LR, Rubin J, Mcmahon RT. VP 16-213 chemotherapy for advanced squamous cell carcinoma and adenocarcinoma of the lung. Cancer Treat Rep 1978; 62: 841.

37. McMahon RT, Ingle JN, Rubin J, Kivos LR. VP 16-213 in advanced squamous cell and adenocarcinoma of the lung. Proc Am Soc Clin Oncol 1978; 19: 396.

38. Langner E, De Teyler R, Taglon, H, Krzesky J, Cuspilatin and VP 16-213 combination chemotherapy in non small cell bronchogenic carcinoma. Phase I-II clinical trial. Proc Am Soc Clin Oncol 1980; 21: 368.

39. Inamori LJ, Danahie JP. Combination chemotherapy in disseminated testicular cancer. The Indiana University experience. Semin Oncol 1979; 6: 87.

40. Cavalli F, Sonntag RW, Brunner KW. Epopodophyllotoxin derivative (VP 16-213) in the treatment of solid tumours. Lancet 1977; ii: 562.

41. Newlands ES, Bagshawe KD. Epopodophyllotoxin derivative (VP 16-213) in malignant teratoma and choriolecancoma. Lancet 1977; ii: 87.

42. Williams SD, Ingham LH, Greco A, et al. VP 16-213 an active drug in germinal neoplasms. Proc Am Soc Cancer Res 1979; 20: 291.

43. Fitzharris BM, Kaye SB, Saverymuttu et al. VP 16-213 as a single agent in advanced testicular tumours. Eur J Cancer 1980; 16: 1193.

44. Cavalli F, Klepp O, Remurd J, et al. A phase II study of oral VP 16-213 in non-seminomatous testicular cancer. Eur J Cancer 1983; 17: 245.

45. Williams SD, Ingham LH, Greco FA, et al. VP 16-213 salvage therapy for refractory germinal neoplasms. Cancer 1980; 46: 2154.

46. Newlands ES, Bagshawe KD. Antitumour activity of the epipodophyllotoxin derivative VP 16-213 (etoposide: NSC-141540) in gestational choriocarcinoma. Eur J Cancer 1980; 16: 401.

47. EORTC Clinical Screening Group: Epopodophyllotoxin VP 16-213 in treatment of acute leukemias, haematocarcinomas, and solid tumours. Br Med J 1975; iii: 199.

48. Mathe G, Schwarzenberg L, Pouillart P, et al. Two epipodophyllotoxin derivatives VM 26 and VP 16-213 in the treatment of leukemias, haematosarcomas, and lymphomas. Cancer 1974; 34: 985.

49. Jungi WF, Senn HJ. Clinical study of the new epipodophyllotoxin derivative 4'-demethylepipodophyllotoxin 9-(4,6-0-ethyldene-β-D-glucopyranoside) (NSC 141540: VP 16-213) in solid tumours in man. Cancer Chemother Rep 1975; 59: 737.

50. Cecil JW, Quaglins JM, Coleman CA, Al-Sarraf M, Thigpen T, Groppe CW. Evaluation of VP 16-213 in malignant lymphoma and melanoma. Cancer Treat Rep 1978; 62: 801.

51. Schechter JP, Jones SR. Myocardial infarction in a 27 year old woman: Possible complication of treatment with VP 16-213 (NSC 141540) mediastinal irradiation or both. Cancer Treat Rep 1975; 59: 887.
subjects were vaccinated with a Dermojet injector; the six remaining volunteers were given vaccine with a 25-gauge needle and tuberculin syringe. No volunteer had received antirabies vaccine previously.

The nuchal muscles of forty-two New Zealand White rabbits were inoculated with 50 rabbit LD50 of a first mouse-brain-passage arctic-fox rabies virus isolate in two separate sites (0.5 ml each side). 8 h later fourteen rabbits were given 1.0 ml of HDCS vaccine intramuscularly (i.m.) into the left forelimb, and fourteen others received 4 i.d. injections of 0.2 ml of vaccine into each limb. The remaining fourteen were used as controls and received no prophylaxis. Each animal was then observed for 12 months for signs of rabies. None of the rabbits had been exposed to rabies previously or had been immunised against the disease.

In both studies whole-virion HDCS rabies vaccine (l'Institut Merieux; lot R 0220; antigenic value 10-8) inactivated with 8-propiolactone was used.

**Titration of Serum Antibody**

All blood samples were titrated for rabies neutralising antibody with the mouse neutralisation test; titres in IU/ml were calculated by reference to the international standard antiserum to rabies virus (Statens Seruminstitut, Copenhagen, Denmark). In addition, the sera were assayed for IgG rabies antibody by enzyme immunoassay (ELISA) using a modification of the method used for the detection of coronavirus antibodies (K. G. Nicholson, H. Prestage, unpublished). Absorbance values were read at 405 nm on a Flow Laboratories Titertek Multiskan spectrophotometer 20, 30, 45, and 60 min after addition of the substrate. Rabies antibody was considered present when the optical-density reading of the test sample was greater than the mean +2 standard deviations of comparable dilutions of 8 negative control sera.

**Lymphocyte Transformation of T and B cell Subpopulations**

An enriched T-lymphocyte population was obtained by passing a thrice-washed mononuclear-cell suspension taken from the top of a Ficoll-Triosil gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) twice through a nylon-fibre column. The cell suspension obtained contained less than 2% B lymphocytes as judged by staining with polyclonal fluorescein-labelled antihuman immunoglobulin reagent. An enriched B-lymphocyte population was obtained by sedimenting rosettes formed between T lymphocytes and sheep red blood cells. Lymphocytes from human cord blood and a non-vaccinated subject were used as controls. HDCS rabies-virus vaccine (l'Institut Merieux; lot R 0220) was exhaustively dialysed against phosphate-buffered saline (PBS) and adjusted to the original volume with PBS for use as antigen. Phytohaemagglutinin (PHA; purified grade, Wellcome Laboratories, Beckenham) was used as control at a concentration of 0.2 mitogenic units/ml. Cultures containing 10 µl of antigen or mitogen and 200 µl of cell suspension containing 2×10⁶ lymphocytes were established in microtitre plates then pulsed with tritiated thymidine and harvested as described previously.

**RESULTS**

Because of the pH indicator in the vaccine, needle inoculation immediately resulted in magenta-coloured skin blebs at each injection site. Vaccination with the dermojet injector was generally quicker, but bleb formation was less satisfactory and in some areas where the skin was especially soft it appeared that all the vaccine had entered the subcutaneous tissue. This method of inoculation was also associated with lower titres of antibody than occurred after needle inoculation (figs. 1 and 2). Substantial titres of neutralising and antirabies IgG antibodies developed in the six subjects who were inoculated with a needle and syringe. By 10 days, neutralising antibody ranged between 1.3 and 8.7 IU/ml (geometric mean titre [GMT] 3.0 IU/ml) and antirabies IgG ranged between 1/345 and 1/1250 (GMT 1/693). Peak titres of virus neutralising antibody were found on day 14 (GMT 14.3 IU/ml), but substantial titres were still present 100 days after vaccination.

The increments (cpm) in lymphocyte transformation are expressed as a ratio to the cpm values of non-stimulated control cultures (table). The results show that T lymphocytes from eight of ten vaccinees were significantly stimulated in vitro 14 to 42 days after vaccination. This blast transformation occurred with cells from three of four people given vaccine by the dermojet injector and from five of the six people inoculated with a needle and syringe. There was no significant difference between the transformation increments of the two groups, and no correlation was found between the transformation increment and the titre of antirabies IgG or
MITOGEN AND ANTIGEN STIMULATION OF PERIPHERAL BLOOD LYMPHOCYTES OBTAINED 0, 10, 14, 21, AND 42 DAYS AFTER VACCINATION OF TEN VOLUNTEERS WITH 0.88 ML OF HDCS RABIES-VIRUS VACCINE

| Lymphocyte stimulation index* | 0 day | 10 days | 14 days | 21 days | 42 days |
|------------------------------|-------|---------|---------|---------|--------|
| PHA Rabies | PHA Rabies | PHA Rabies | PHA Rabies | PHA Rabies | PHA Rabies |
| B | T | B | T | B | T | B | T | B | T | B | T | B | T | B | T | B | T | B |
| Inoculated by needle and syringe | a. | 79 | 3 | 1 | 1 | 86 | 1 | 1 | 1 | 58 | 3 | 1 | 1 | 77 | 1 | 35 | 1 | 94 | 1 | 39 | 1 |
| b. | 91 | 1 | 1 | 1 | 73 | 2 | 1 | 1 | 80 | 1 | 1 | 2 | 82 | 2 | 27 | 1 | 98 | 1 | 61 | 1 |
| c. | 47 | 4 | 1 | 1 | 82 | 2 | 1 | 1 | 92 | 1 | 29 | 1 | 76 | 1 | 39 | 1 | 168 | 1 | 36 | 1 |
| d. | 58 | 2 | 1 | 1 | 75 | 1 | 1 | 1 | 87 | 1 | 2 | 1 | 102 | 1 | 28 | 1 | 75 | 1 | 14 | 1 |
| e. | 53 | 2 | 1 | 1 | 74 | 1 | 1 | 1 | 87 | 1 | 2 | 1 | 102 | 1 | 28 | 1 | 75 | 1 | 14 | 1 |
| f. | 102 | 1 | 1 | 1 | 99 | 1 | 1 | 1 | 86 | 1 | 2 | 1 | — | — | — | — | 105 | 1 | 48 | 1 |
| Inoculated by dermojet | g. | 54 | 2 | 1 | 1 | 68 | 1 | 1 | 1 | 87 | 1 | 1 | 1 | 49 | 4 | 1 | 1 | 81 | 1 | 2 | 1 |
| h. | 83 | 2 | 1 | 1 | 60 | 1 | 1 | 1 | — | — | — | — | 71 | 1 | 48 | 1 | 104 | 1 | 31 | 1 |
| i. | 95 | 3 | 1 | 1 | 82 | 2 | 1 | 1 | 101 | 1 | 24 | 1 | 83 | 1 | 52 | 1 | 92 | 1 | 20 | 1 |
| j. | — | — | — | — | 79 | 1 | 1 | 1 | 93 | 1 | 1 | 1 | 92 | 1 | 11 | 1 | 104 | 1 | 22 | 1 |
| Unvaccinated | k. | 86 | 1 | 1 | 1 | 89 | 1 | 1 | 1 | 71 | 2 | 1 | 1 | 99 | 1 | 1 | 1 | 69 | 1 | 1 | 1 |
| Cord blood | l. | 105 | 1 | 1 | 1 | 91 | 1 | 1 | 1 | 95 | 2 | 1 | 1 | 105 | 1 | 2 | 1 | 77 | 1 | 1 | 1 |

*Lymphocyte stimulation index = cpm in stimulated culture / cpm in unstimulated culture. A value of >3 represents significant lymphocyte stimulation. PHA = phytohaemagglutinin.

DISCUSSION

Considerable evidence has accumulated from studies in Britain, France, and Germany that the administration of HDCS vaccine by the intradermal route is successful postexposure treatment of man. In these animals, forelimb paralysis developed within 13 to 38 days of infection and progressed to complete paralysis and death 2 to 7 days later. Postexposure treatment with a single dose of HDCS vaccine reduced the mortality significantly; the administration of 1·0 ml of vaccine i.m. gave significant (p = 0.018, Fisher's exact test) but incomplete protection, two of fourteen animals developing paralysis and dying after incubation periods of 13 and 20 days. None of fourteen rabbits died after receiving 4 i.d. inoculations of 0·2 ml of the vaccine in each limb (p = 0.0006).

Vacines (eight of ten) as was found in the previous study, but with only a quarter of the volume of vaccine. Clearly, if high titres of neutralising antibody and a cell-mediated response are both important for protection, the present study suggests that they can be obtained equally well with much smaller quantities of vaccine than are used at present. We further showed that rabbits could be completely protected by injecting 4 × 0·2 ml doses of HDCS vaccine intradermally 8 h after intranuchal infection with street-rabies virus. By contrast, none of the fourteen controls (64%) died from rabies with incubation periods of 13 to 38 days (mean 22 days). Opponents to the administration of rabies vaccine by the i.d. route claim that it is technically difficult, especially in the elderly and the very young. Nevertheless, many vaccines are routinely administered by the i.d. route with apparent success and considerable financial savings. There seems to be ample experimental evidence to justify a postexposure study of HDCS vaccine administered by the i.d. route; we believe that this would be both ethical and potentially of great importance for developing countries.

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REFERENCES

1. Bahmanyar M, Fayaz A, Nouar-Salehi S, Mohammad M, Kopenhagen H. Successful protection of humans exposed to rabies infection. Postexposure treatment with the new human diploid cell rabies vaccine and antirabies serum. JAMA 1976; 236: 2751-54.
2. Kuwert EK, Marcus I, Werner J, et al. Post-exposure use of human diploid cell culture rabies vaccine. In: Joint WHO/IABS Symposium on the standardization of cell substrates for the production of virus vaccines. In: Perkins FT, Regamey RH, eds. Developments in biological standardization. Basel: S. Karger, 1977; 273-86.
3. Anderson LJ, Sikes RK, Langkamp CW, Mann JM, Smith JS, Winkler WG, Dench MW. Postexposure trial of a human diploid cell strain rabies vaccine. J Infect Dis 1980; 142: 135-38.
4. Nicholson KG, Turner GS, Aoki FY. Immunization with a human diploid cell strain rabies virus vaccine: two year results. J Infect Dis 1978; 137: 275-54.
5. Nicholson KG, Turner GS. Studies with human diploid cell strain rabies vaccine and human rabies immunoglobulin in man. In: Hennessen W, Regamey RH, eds. Joint WHO/IABS Symposium on the standardization of rabies vaccines for human use produced in tissue cultures (Rabies III). Marburg/Lahn 1977. Developments in biological standardization. Basel: S. Karger, 1978; 40: 115-20.

References continued overleaf
INSULIN was first used therapeutically in 1922. Its use has transformed the management of diabetes and it is now administered to about 4 million diabetics throughout the world. Although the frequency of side-effects is relatively low, efforts have been made over the years to improve the quality and extend the range of insulin preparations. The pace of these changes has accelerated during the past decade. Introduction of high-purity insulin and of preparations of insulin from a single species of animal have been followed by developments of two kinds: the availability for therapy of insulin containing the aminoacid sequence of the natural human hormone and the construction of continuous-delivery systems to administer insulin under conditions that more closely mimic its natural secretion in the body. The changes in quality and type of insulin available have posed problems of nomenclature.

Recognition that the molecular structure of insulin can influence tolerance to, and the side-effects of, therapy led to the introduction in the British Pharmacopoeia (BP) in 1975 of a requirement that all formulations be labelled with the species of origin. At that time, the BP contained no monograph for bulk insulin, but the marketing of a variety of species of origin. This has had several consequences. First, the monograph required a title and "Insulin" was taken. Had the title been "Purified insulin" or similar, to draw a distinction with crystalline insulin, the problem might have arisen of a name for material of yet higher quality (already available as "monocomponent" or "pro-insulin-free" preparations). This is one reason why a monograph for a drug substance rarely contains any qualifying adjective indicating degree of purity. However, the title "Insulin" had been for many years a synonym for Insulin Injection, and it was necessary to delete it as an alternative name for that preparation. Second, a unique situation arose in that the monographs for formulations contained to specify a minimum potency of 23 IU/mg for the insulin used, against the higher figure in the bulk monograph, thus allowing insulin not of BP quality to be incorporated into BP formulations. This was the result of decisions not to delete conventional formulations, satisfactory for many patients, from the pharmacopoeia and not to add a set of separate monographs for formulations containing the BP grade of insulin, which would require additional titles. Another factor was involved in that control of one of six of the nine insulin formulations is exerted by monographs in the European Pharmacopoeia. For these, no unilateral change in the requirements can be made by a national authority, but revision must await agreement by the European Pharmacopoeia Commission, with a further lapse of time before adoption. This factor illustrates the point that pharmacopoeial decisions in the United Kingdom, including those involving nomenclature, cannot be taken without considering the international constraints that apply and the international implications that may result.

**HUMAN INSULIN**

During the past eighteen months another aspect of insulin nomenclature has been discussed by the British Pharmacopoeia Commission and its committees concerned with nomenclature and with hormones. This arose from availability, for testing and clinical trial, of insulin possessing the structure of the natural human hormone, the prospect of its wider use, and the eventual need for a monograph for it. That one method of production of such insulin uses genetic-engineering techniques suggests that the wider implications must be considered. Thus, in the following discussion, it should be remembered that the final outcome should, so far as possible, set a pattern that can be applied to other hormones and therapeutic substances (such as growth hormone and interferons) obtained by novel techniques.

The structure of human insulin was established in 1963 and was found to differ from that of the porcine hormone only in the presence of threonine in place of alanine at position 30 of the B chain. The total synthesis of insulin having the human sequence was achieved in an elegant manner by workers at Ciba Geigy in 1974, but the process is not economically viable under present conditions. This material...