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Public Health

MULTISITE INTRADERMAL ANTIRABIES VACCINATION

Immune Responses in Man and Protection of Rabbits Against Death from Street Virus by Postexposure Administration of Human Diploid-Cell-Strain Rabies Vaccine

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Summary Lymphocyte transformation, production of neutralising antibody, and the development of antirabies IgG antibody were studied in ten healthy volunteers in response to 0·8 ml of human diploid-cell strain (HDCS) rabies vaccine administered on one occasion in divided doses in 8 intradermal (i.d.) sites. All ten volunteers rapidly developed substantial titres of rabies antibody, and eight of the ten had T lymphocytes that were immunologically stimulated by HDCS rabies-virus antigen. Postexposure treatment with 0·8 ml of HDCS vaccine given at 4 i.d. sites completely protected fourteen rabbits from death by street virus. The results suggest that in developing countries patients could be protected with small volumes of potent tissue-culture vaccine administered intradermally shortly after exposure.

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 1 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.[4-7] 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

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subjects were vaccinated with a Dermojet injector;[8] 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 LD_{50} 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 ß-propiolactone was used.

Titration of Serum Antibody

All blood samples were titrated for rabies neutralising antibody with the mouse neutralisation test;[9] 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[10] (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.[11] The cell suspension obtained contained less than 2% B lymphocytes as judged by staining with polyvalent fluorescein-labelled antihuman immunoglobulin reagent. An enriched B-lymphocyte population was obtained by sedimenting rosettes formed between T lymphocytes and sheep red blood cells.[11] 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 2×10^5 lymphocytes were established in microtitre plates then pulsed with tritiated thymidine and harvested as described previously.[7]

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.

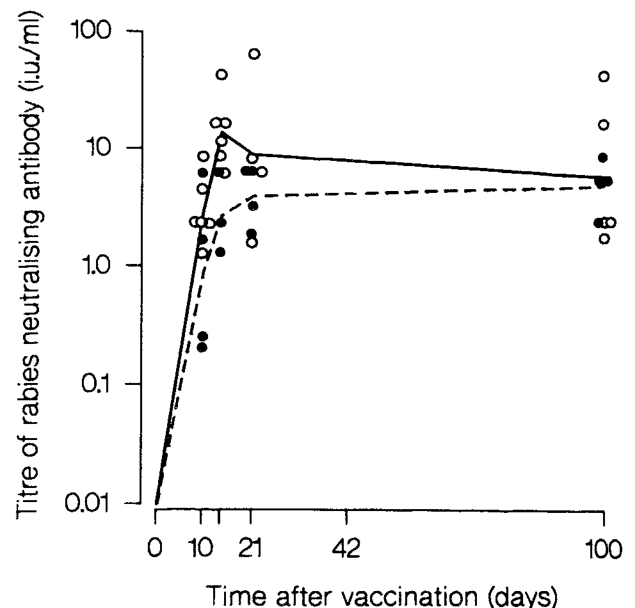

Fig. 1—Individual and geometric mean titres of rabies neutralising antibody after inoculation with needle and syringe (open circles, solid line) and dermojet injector (closed circles and broken line).

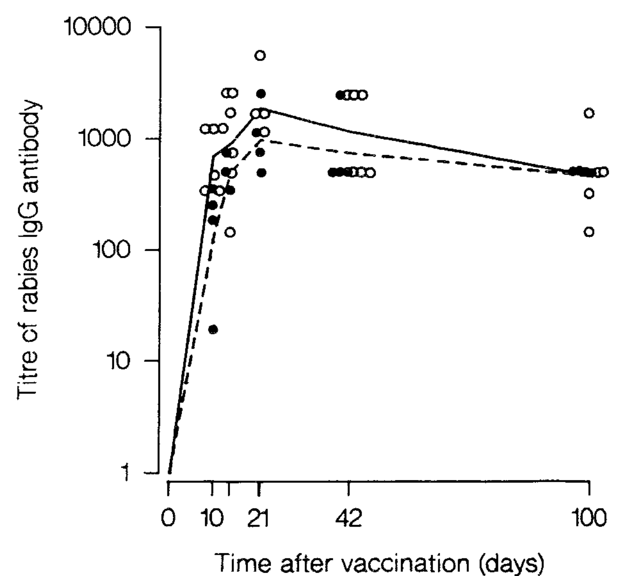

Fig. 2—Individual and geometric mean titres of rabies IgG antibody (established by ELISA) after inoculation with needle and syringe (open circles, solid line) and dermojet injector (closed circles and broken line).

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 0·8 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	
—		T	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	2	1	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	95	1	1	1	—	—	2	—	—	—	—	—	82	1	1	1
	e.	63	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 injection	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	1	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 = $\frac{\text{cpm in stimulated culture}}{\text{cpm in unstimulated culture}}$. A value of >3 represents significant lymphocyte stimulation. PHA = phytohaemagglutinin.

neutralising antibody. None of the enriched B-cell cultures underwent blast transformation in response to the rabies antigen.

Nine of fourteen rabbits developed paralysis and died after infection with 50 rabbit LD_{50} of street-rabies virus. 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).

DISCUSSION

Considerable evidence has accumulated from studies in Britain,[4-7,12,13] France,[14] and Germany[15,16] that the administration of HDCS vaccine by the intradermal route is followed by substantial titres of virus-neutralising antibody with occasional mild local and systemic reactions. It has also been shown that 4 or 8 doses of 0·1 ml given in separate sites on a single occasion rapidly induce high titres of antibody.[6,7] The present report confirms these observations and shows in addition that the early production of virus-neutralising antibody is accompanied by high titres of antirabies IgG. This early IgG response may be most important, for it is now well established that neutralising antibody of the IgG class, unlike IgM neutralising antibody, confers protection on animals challenged with rabies[17] and may be the key to successful postexposure treatment of man.

The possible role of cell-mediated immunity in rabies infection is poorly understood, but it too may be an important component of the host's immune response. We have reported that transformation of lymphocytes occurred with cells taken from eight of ten vaccinees after the first 4 × 1 ml doses of an established postexposure regimen for HDCS vaccine.[7] In the present study we separated the lymphocyte subpopulations and have shown that the blast transformation is a T-cell response. Furthermore, it occurred in the same proportion of vaccinees (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, nine 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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British Pharmacopoeia Commission

THE NOMENCLATURE OF INSULIN

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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[1] 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 purified insulins made such a monograph desirable, and it appeared in the BP 1980.[2] The monograph covers insulin of either porcine or bovine origin that has been purified beyond the stage of conventional crystalline insulin. 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 arise 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 continued 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 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.

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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 1960[3] 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,[4] but the process is not economically viable under present conditions. This material

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