E-ROSETTE FORMING CELLS AND HUMORAL ANTIBODY TITRES IN HUMANS AFTER VACCINATION WITH THREE DIFFERENT INACTIVATED INFLUENZA VIRUS VACCINES A/USSR/92/77 (H1N1)

S. SCHMIDT, J. SÜSS, H. OEHRING, J. SCHMIDT

Institute of Medical Microbiology, Friedrich Schiller University of 69 Jena, German Democratic Republic

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Summary. — The number of E-rosette forming cells and the serum haemagglutination inhibition (HI) antibody titres were examined in 37 volunteers immediately before and 14, 28, 35 and 63 days after immunization with three inactivated influenza virus vaccines A/USSR/92/77 (H1N1) — NIB 6 and in 11 non-vaccinated controls. From the former, 10 volunteers were immunized with 1000 haemagglutinin (HA) IU per dose, 11 volunteers with the NIB 6 adsorbate vaccine (340 HA IU/dose) and 16 volunteers with a bivalent vaccine composed of 180 HA IU/dose NIB 6 and 180 HA IU/dose of influenza virus A/Bangkok X-73 (H3N2). The percentage of E-rosette forming cells was decreased in all vaccinated volunteers 14 days after vaccination; later on the values reached normal level of non-vaccinated controls or of subjects before vaccination. The number of E-rosette forming cells was in correlation with the applied virus vaccine dose, i.e. for the 1000 HA IU/dose: $29.95 \pm 11.74\%$, $p < 0.001$ and for the 340 HA IU/dose: $47.75 \pm 11.15\%$, $p < 0.005$; however, after administration of 180 HA IU/dose of NIB 6 in the bivalent vaccine, the value $58.65 \pm 11.5\%$ was not significantly decreased in comparison to non-vaccinated donors. The serum HI antibody titres reached the highest level 14 days after vaccination and remained constant during the next 6 weeks. There was a correlation between decreased E-rosette values and increased serum antibody titres ($p < 0.05$).

The current study indicates that the number of E-rosette forming cells may serve as a further laboratory criterion for controlling the effect of inactivated influenza virus vaccines on the immune system of man.

Key words: *E-rosettes, influenza vaccination, inactivated vaccine, HI antibodies*

Introduction

Induction of immunological resistance against influenza virus infections in humans is the aim of the worldwide used immunization with inactivated or live influenza virus vaccines. The commonly available influenza virus

vaccine is an inactivated one and this will be so in coming years (Palese, 1980). It should be pure, immunologically potent, nonreactive and possess an adequate antigenicity (Stuart-Harris, 1981). In response to such a vaccine, many factors were found changing in the immunological system; especially the haemagglutination inhibition (HI) antibody titre had been widely accepted as protection index (Hobson *et al.*, 1972), but also antibodies against neuraminidase may change after application of a killed influenza virus vaccine (Sandow *et al.*, 1980).

The role of cell-mediated immune response after vaccination is not yet clear. More simple laboratory criteria are needed for determination of the effect of vaccine or their influence on the immune system. Such a criterion may be the number of E-rosette forming cells after application of live influenza virus vaccines (Rudenko *et al.*, 1978). The capacity of human T cells to bind sheep red blood cells (SRBC) has become a standard tool for their delineation in peripheral blood or in other cell suspensions (Lay *et al.* 1971; Fröland, 1972; Jondal, 1976).

This paper presents results of determination of the number of E-rosette forming cells and of the serum antibody titres by haemagglutination inhibition test (HIT) and by ELISA-technique in volunteers after vaccination with three batches of inactivated experimental influenza virus vaccine containing different amounts of virus antigen (A/USSR/92/77, H1N1). In contrast to others (Rudenko *et al.* 1978), we found a significant dose-dependent response in the number of E-rosette forming cells. We have also determined a correlation between the antigen content in vaccines, the number of E-rosette forming cells and the serum antibody titres in post-vaccination period. In contrast to others (Rudenko *et al.*, 1978), we believe that the number of E-rosette forming cells may be used as an additional laboratory criterion evaluating the immune response also to the killed vaccines and not only to the live ones (Rudenko *et al.*, 1978) or to wild influenza virus strains (Savitsky *et al.*, 1978).

Materials and Methods

Volunteers and vaccines. Healthy students, 18—23 year old, were vaccinated with 0.5 ml o inactivated, egg-grown influenza virus vaccine (experimental batches) as follows: n — 10 volunteers received the Al $(OH)_3$ adsorbed vaccine NIB 6, purified by zonal centrifugatio of recombinant virus A /USSR/92/77 × A/PR/8/34 (H1N1), in a dose of 1000 HA IU; — 11 volunteers were vaccinated with the adsorbate NIB 6 vaccine receiving 340 HA IU per dose; — 16 students received a bivalent vaccine containing 180 HA IU of NIB 6 (H1N1) per dose and the influenza A/Bangkok/1/79 × A/PR/8/34 (H3N2) recombinant strain as second component in the same virus antigen concentration.

Age-matched 11 non-vaccinated students served as controls. Venous blood was obtained from each volunteer immediately before and 14, 28, 35 and 63 days after vaccination including the non-vaccinated volunteers.

Preparation of lymphocytes and E-rosettes. Preparation of lymphocytes was performed as described (Schmidt *et al.*, 1981). Briefly, lymphocytes were prepared according to Bøyum (1968) in lymphocyte separation medium (Litton Bionetics, Kensington, U.S.A.) by centrifugation at 400 × g (for 35 min at room temperature). The mononuclear cell fraction was washed twice in culture medium (Eagle's MEM) and diluted to a density of 5×10^6/ml. 200 µl of this cell suspension were carefully mixed with SRBC (1×10^8/ml; 200 µl) and incubated (30 min, 4 °C).

After centrifugation (2 min, 200 g) the pellet was allowed to stand overnight (4 °C). Enumeration of the E-rosette forming cells in permanent preparations was performed according to Helbig and Lehmann (1978) by a modified method (Schmidt *et al.*, 1981). Each test sample was prepared three times. 300 lymphocyte-like cells were counted and cells binding 3 or more SRBC were considered positive.

Serum antibody titration. Sera were tested against the homologous virus strain NIB 6 (H1N1) in HIT after treatment with potassium periodate (Starke, 1968) and by the ELISA-technique.

Statistical analysis. Statistical differences were evaluated by means of the Student's t-test (Richterich, 1965).

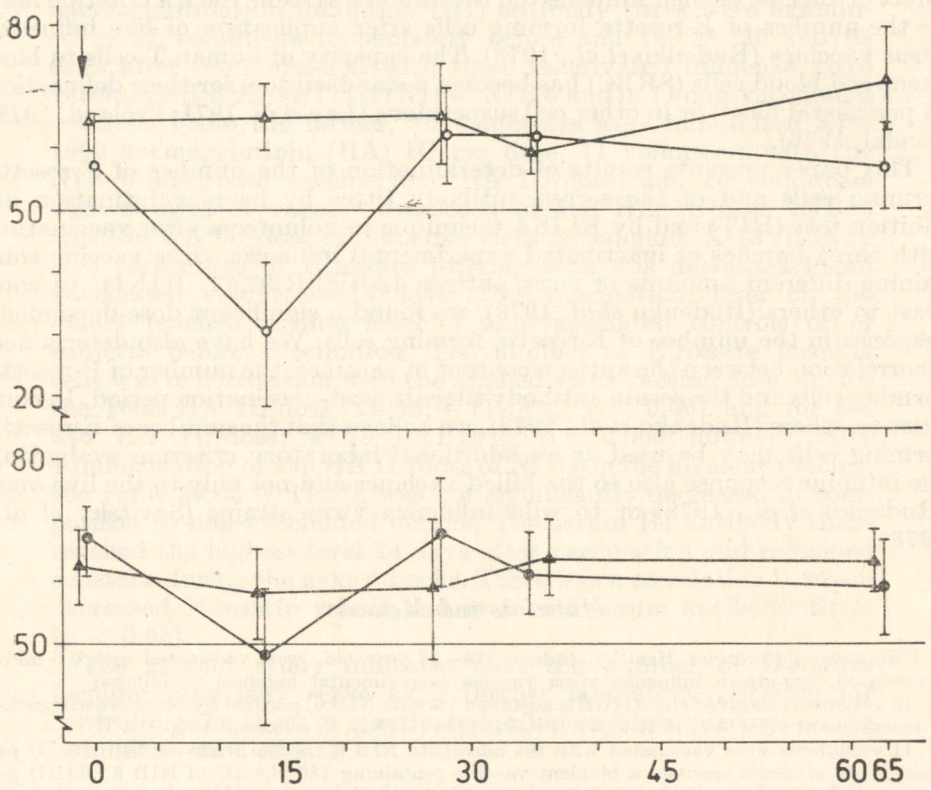

Fig. 1.

E-rosette forming cells in the mononuclear cell fraction of volunteers after vaccination with different inactivated influenza virus vaccines A/USSR/92/77 (H1N1)

Symbols: (△——△) non-vaccinated controls; (▲——▲) bivalent vaccine: 180 HA IU A/USSR/92/77 (H1N1) and 180 HA IU A/Bangkok X-73 (H3N2); (●——●) 340 HA IU of A/USSR/92/77 adsorbate vaccine; (○——○) 1000 HA IU of A/USSR/92/77 zonal centrifuged vaccine. Abscissa: days post vaccination; ordinate: per cent of E-rosette forming cells in the mononuclear cell fraction. Horizontal bars: ± SD.

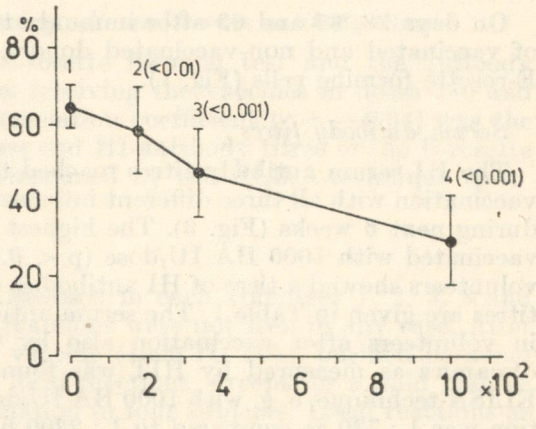

Fig. 2.

Percentage of E-rosette forming cells in the mononuclear cell fraction of vaccinated volunteers 14 days after vaccination as related to antigen concentration in 3 experimental batches of influenza virus vaccines A/USSR/92/77 (H1N1) in comparison to non-vaccinated controls (mean values ± S. D.). For details see Fig. 1. and Results. Abscissa: HA IU × 10^2 per dose; ordinate: per cent of E-rosette forming cells. 1 — Non vaccinated controls; 2 — — Bivalent vaccine; 3, 4 — Influenza virus A/USSR/92/77 vaccines; (p values as compared to controls in brackets)

Results

E-rosette forming cells after vaccination

Fig. 1 shows the time course of the relative number of E-rosette forming cells in three groups of vaccinated volunteers and in non-vaccinated controls. There was a strong decrease of the percentages of E-rosette forming cells 14 days after vaccination in association with the amount of the applied virus antigen (after 1000 HA IU/dose: 57.88 ± 7.7% before and 29.95 ± ± 11.74% after vaccination, $p < 0.001$; after 340 HA /Uldose: 66.17 ± ± 5.53% before and 47.75 ± 11.15% after vaccination, $p < 0.005$) However, after administration of 180 HA IU/dose the slight decrease in (E-rosette forming cells to 58.65 ± 11.5% after vaccination was not significant n.s.) as compared to 62.19 ± 6.6% before vaccination; alternatively, in non-vaccinated controls the difference between 67.17 ± 5.37% on the same day before vaccination and 64.78 ± 5.45% on the 14th day was n.s. The differences in the percentage of E-rosette forming cells in the three groups of vaccinated donors on the 14th day after vaccination are significant in comparison to in non-vaccinated donors (Fig. 2).

Table 1. Geometric mean values of serum antibody titres after vaccination with three influenza virus (H1N1)-NIB 6 vaccines containing different amounts of haemagglutinin units

Vaccination dose HA IU	Geometric mean antibody titres (HIT)		
	before vaccination	14 days after vaccination	increase
1000	1 : 11.3	1 : 59.7	5.3
340	1 : 29.9	1 : 68.1	3.1
180 (+ 180 HU IU H3N2)	1 : 24.4	1 : 40.9	1.7

On days 28, 35 and 63 after immunization the mononuclear cell fraction of vaccinated and non-vaccinated donors revealed the same percentage of E-rosette forming cells (Fig. 1).

Serum antibody titres

The HI serum antibody titres reached the highest level by 14 days after vaccination with all three different influenza vaccines and remained constant during next 6 weeks (Fig. 3). The highest increase was found in volunteers vaccinated with 1000 HA IU/dose ($p < 0.005$). After vaccination nearly all volunteers showed a titre of HI antibodies of 1 : 32 or more. The exact mean titres are given in Table 1. The serum antibody titres have been determined in volunteers after vaccination also by the ELISA-technique; the same dynamics as measured by HIT was found with the much more sensitive ELISA-technique, e. g. with 1000 HA IU/dose the mean titre before vaccination was 1 : 730 as compared to 1 : 2300 by 14 days after vaccination ($p < < 0.02$).

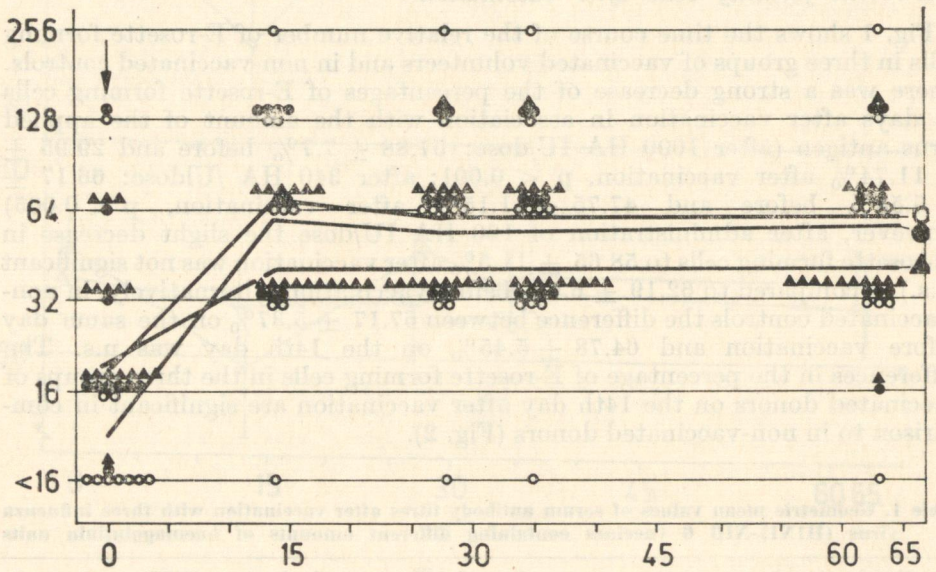

Fig. 3.

Time-course of HI antibody titres in sera of volunteers after vaccination with different inactivated influenza virus vaccines A/USSR/92/77 (H1N1), tested to homologous influenza virus antigen Symbols: (▲) 180 HA IU of influenza virus A/USSR/92/77 (H1N1) and 180 HA IU of influenza virus A/Bangkok X-73 (H3N2); (○) 340 HA IU of the A/USSR/92/77 adsorbate vaccine; (●) 1000 HA IU of the A/USSR/92/77 zonal centrifuged vaccine. Abscissa: days post-vaccination; ordinate: HI antibody titre.

Correlation between E-rosette forming cells and serum antibody titres

We compared the results of E-rosette forming test and the antibody titres (HIT, ELISA) in volunteers receiving the vaccines in doses 340 and 1000 HA IU, respectively. The correlation coefficient ($r = -0.34$) was the same either for the E-rosette values and HI-antibody titres of the E-rosette values and the antibody-titres determined by the ELISA-technique ($p < < 0.05$).

Reactions to the vaccine

Reactions to the vaccine were assessed in each volunteer 1, 2, 3, 8 and 28 days after inoculation. Strong reactions were not seen in any case. After immunization with the high-dose vaccine (1000 IU HA), purified by zonal centrifugation, volunteers had no local reactions, except for a mild malaise during the 3 first days after vaccination in four subjects. Local reactions as redness and swelling up to 4 cm in diameter were found after application of the two other types of vaccines, but no systemic reactions were seen.

Percentage of lymphocytes in the peripheral blood and the number of isolated mononuclear cells

There were $37.3 \pm 12.7\%$ lymphocytes in the peripheral blood. Before vaccination. Vaccination did not influence this value, regardless which vaccine had been used. Slight differences among values in the 4 groups of volunteers were not statistically significant. Neither the number of isolated white blood cells per ml as determined by Bøyum's technique differed substantially before and after vaccination (1000 HA IU/dose: $1.4 \pm 0.26 \times \times 10^6$/ml before and $1.62 \pm 0.58 \times 10^6$/ml after vaccination; 340 HA IU/ /dose: $1.10 \pm 0.32 \times 10^6$/ml before and $1.19 \pm 0.40 \times 10^6$/ml after vaccination; 180 HA IU/dose: $1.12 \pm 0.25 \times 10^6$/ml before and $1.18 \pm 0.43 \times \times 10^6$/ml after vaccination).

Discussion

At 14 days after vaccination of young volunteers with three experimental batches of inactivated influenza virus vaccines A/USSR/92/77 (H1N1) a strong transient decrease of the number of E-rosette forming cells was found in the mononuclear cell fryction separated by the Bøjum's technique. The decrease of T cells was related to the concentration of virus antigen in the vaccines; it was very intensive after application of 1000 and 340 HA IU/dose, respectively ($p < 0.001$). In comparison to non-vaccinated donors it was also statistically significant after application of the bivalent vaccine containing 180 HA IU of NIB 6 ($p < 0.01$). Such a significant decrease of the number of T-lymphocytes is a sign of immunosuppression. There is a high level of evidence that an influenza virus infection with wild or attenuated strains leads to a transient lymphopenia (Criswell *et al.*, 1975; Scheinberg *et al.*, 1976; Dolin *et al.*, 1977; Rudenko *et al.*, 1978), but only Kurokawa *et al.* (1975) described leukopenia and lymphopenia after application of an in-

activated influenza virus vaccine in mice. Recently description by Faguet (1981) is also in accordance with the presented results. After application of a killed influenza virus vaccine (A/New Jersey/76 [Hsw1N1]) in man he found a very early T and B lymphocytopenia and an increased level of null cells, in response to a single vaccine dosage only. In contrast to our results, Rudenko *et al.* (1978) reported no influence of the applied inactivated vaccine on the number of T cells. We believe that the suppression of the number of T cells after application of inactivated influenza virus vaccine is an effect of virus concentration and not of the vaccine purity. The most intensive suppression of the number of T cells was found in volunteers vaccinated with the virus highly purified by zonal centrifugation and used in the highest antigenic concentration.

In most volunteers tested after vaccination with the three different vaccines, HI antibody titres ranged between 1 : 32/64; this value has been commonly accepted for the minimal protection level against infection (Hobson *et al.*, 1972). Antibody titres reached optimal values 14 days after immunization with the vaccine A/USSR/92/77 (N1N1), at the same time the E-rosette numbers were strongly decreased, the correlation coefficient r being -0.34 ($p < 0.05$). May be this effect is due to the balance between cell-mediated and humoral immune reactions after vaccination with the influenza virus vaccines. Precise mechanism why the T lymphocyte number has decreased remains unknown.

We think that the number of E-rosette forming cells may serve as a further laboratory criterion for the efficiency of inactivated influenza virus vaccines in humans.

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