# SOME PROBLEMS OF HUMORAL SMALLPOX IMMUNITY

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Since the character of skin response in revaccination against smallpox has proven an unreliable criterion in judging insusceptibility (Mastyukova et al, Card, Pishunova, Stevenson), in recent years the needs of using determination of antibody levels for this purpose (Makauskas, Nemmeyanova) has been indicated. Most specialists (Downie, McCarthy, and Downie) hold that antismallpox immunity is related to neutralization by antibodies, while others (Mastyukova, Nemmeyanova) believe that an important index of immunity against smallpox is the level of antihemagglutinins. A gradual decrease in the antibody level takes place following inoculation (Collier and Schonfeld); vaccinal immunity is lost and the index of inoculability rises (Cross).

Data on the ratio and duration of the presence of various antibodies following inoculation is highly contradictory. According to the data of most investigators (Mineyeva, Downie and Hobday) neutralizing antibodies are found in sera considerably longer than are antihemagglutinins. Complement-fixing antibodies do not appear in all cases and quickly disappear (McCarthy and Downie). The kind of skin reaction upon inoculation as a function of the original antibody level has been little studied. Some authors (Nemmeyanova, Skrotskiy et al) assert that the quantity of antibodies determines the clinical course

of the inoculation, while others (Mineyeva) deny this dependence.

Presented in this report are results of the study of certain debatable and little studied problems of humoral anti-smallpox immunity. The recording of results of revaccination of persons with dermovaccine prepared at the Institute of Epidemiology and Microbiology imeni Gamaleya was carried out on the third, fifth, and seventh day and these results were found to be positive if the skin reaction followed the primary type, and negative, if it was absent or followed the rapid type. Sera from inoculated patients was obtained by venapuncture. The hemagglutination-inhibition reaction followed the accepted method at room temperature, and contact of the serum containing two hemagglutinating antigen units continued for 45-60 minutes. The component-fixation reaction was performed according to the usual method, and complement fixation was carried out at 4° for 18 hours. The neutralization reaction followed the method of McCarthy and Downie. Contact of equal volumes of a mixture of various five-fold dilutions (up to 1:127) of sera studied with a constant dose of smallpox virus (smallpox-forming unit per ml) was carried out at 35° for 2-2½ hours.

During the first series of experiments, we compared the ratio between the antihemagglutinins and neutralizing antibodies in the sera of 233 revaccinated persons (Table 1). The interval of time between the last inoculation and blood sampling ranged from 15-20 days to 12 months.

TABLE 1

Ratio Between Antihemagglutinins and Neutralizing Antibodies

| (a) Титр антигемагглютининов | (b) Число сывороток | | (d) Число сывороток с различными титрами нейтрализующих антител (в %) | | | | |
|---|---|---|---|---|---|---|---|
| | (c) абс. | % | 0[1] (e) | Ц[2] (f) | 1:5 | 1:25 | >1:128 |
| <1:10 | 31 | 13,3 | 6,4 | 25,8 | 19,4 | 35,5 | 12,9 |
| 1:10 | 70 | 30 | 0 | 12,9 | 20 | 50 | 17,1 |
| 1:20—1:40 | 135 | 58 | 0 | 8,7 | 29,7 | 30,4 | 32,2 |
| 1:80—1:160 | 17 | 7,3 | 0 | 0 | 17,65 | 17,65 | 64,7 |
| (g) Всего | 233 | | 0,... | | 2... | 36 | 27,5 |

[1] - 0 = undiluted serum reduced the number of "potmarks" compared to the control down to less than 50 %. [2] Ц = undiluted serum.

LEGEND: a) titer of antihemagglutinin; b) number of sera; c) absolute; d) number of sera with titers of neutralizing antibodies (in percentage) is listed; e) 0[1]; f) Ц[2]; g) total.

The results of the experiments showed that among titers of the reactions studied there was usually a direct dependence observed. For example, the number of sera neutralizing the virus at the dilution ≥ 1:5 was 87.1, 91.3, and 100 %, respectively, for sera with the following

antihemagglutinin titers -- 1:10, 1:20-1:40, and 1:80-1:160. However, the absence of antihemagglutinins in the 1:10 titer still did not signify that neutralizing antibodies was totally lacking in these titers: these entities were contained at a 1:5 titer in 67.8 % of such sera and were totally lacking only in 6.4 % of sera studied.

The presence and level of antihemagglutinins, neutralizing and complement-fixating antibodies sampled at the same periods of time following revaccination. It was established that if the antihemagglutinin titer was less than 1:10, complement-fixating antibodies in the 1:10 titer were also absent, while at the 1:10-1:40 titers, they were determined in 35.4 % of sera studied, and were present at titers of 1:10-1:40 in 100 %, in sera having high antihemagglutinin levels (1:80-1:160).

In a comparative study of the level of antihemagglutinins and neutralizing antibodies in sera sampled at different times following inoculation (Table 2), it was established that in 15-20 days after revaccination, 92.5 % of the sera contained antihemagglutinins in titers $\geq$ 1:10; in 1-3 months the number of negatively reacting sera increased to 30/5 %; in following periods (as much as up to 12 months) the number of positive sera remained approximately at the same level (87.8-88.2 %), but markedly decreased in 1.5-2.5 years (74.35 %). Although the mean antihemagglutinin titer during the course of the year remained at the same level (1:20), a distinct trend to decreased antibody content was observed. In 1.5-2.5 years following revaccination 60.25 % of the sera did not contain these antibodies or contain them at a low titer (1:10). A similar regularity was observed with respect to neutralizing antibodies. In addition, it was established that both upon a positive as well as a negative skin reaction the extent of antibody level decrease was entirely the same.

In other investigations, we determined the effect of the interval between inoculations and their frequency on the level of humoral immunity (Table 3). It was established that the mean level of neutralizing antibodies is a direct function of the frequency of revaccination and the total number of inoculations. This level reached high values (1:25) even after the first inoculation in a group of persons revaccinated every six months. After the last two revaccinations, the level of neutralizing antibodies increased markedly and averaged after four inoculations $\geq$1:125. It must be noted that not one of the 54 sera investigated within this group of individuals contained neutralizing antibodies at a titer less than 1:5. A similar irregularity was also noted after a once repeated revaccination at an interval of a year. For the case of a single revaccination, in two years 32 % of the sera contained neutralizing antibodies only in the naked serum and their average titer was lower than other groups of inoculated persons (1:5).

A clearly pronounced dependence of the antihemagglutinin level on the frequency of revaccinations and the total number of inoculations was lacking. This was due to the fact that in most cases persons with

TABLE 2

Antibody Level at Different Times Following Last Revaccination

| Время после последней ревакцинации | (b) Число исследованных сывороток | (c) Число сывороток с данным титром (в %) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (d) антигемагглютининов | | | | | | (f) нейтрализующих антител | | | | |
| | | <1:10 | 1:10 | 1:20 | 1:40 | >1:80 | в среднем (e) | верхний титр (g) | 1:5 | 1:25 | >1:125 | в среднем (h) |
| 15—20 дней (i) | 189/100 | 7,5 | 19 | 28 | 33,3 | 12,2 | 1:20 | 13 | 19 | 35 | 33 | 1:25 |
| 1—3 месяца (j) | 36/9 | 30,5 | 16,7 | 22,2 | 25 | 5,5 | 1:20 | 0 | 22,2 | 55,6 | 22,2 | 1:25 |
| 4—6 месяцев (k) | 49/25 | 12,2 | 22,5 | 30,6 | 24,5 | 10,2 | 1:20 | 4 | 40 | 32 | 24 | 1:25 |
| 7—12 » (l) | 51/38 | 11,8 | 33,3 | 27,4 | 21,6 | 5,9 | 1:20 | 5,8 | 23,7 | 39,5 | 21 | 1:25 |
| 1½—2½ года (m) | 78/60 | 25,65 | 34,6 | 25,65 | 11,5 | 2,6 | 1:10 | 20 | 43,3 | 25 | 11,7 | 1:5 |

The numerator stands for the number of sera investigated in the hemagglutinin-inhibition reaction, the denominator stands for the number of sera investigated by the neutralization reaction.

LEGEND: a) time following last revaccination; b) number of sera investigated; c) number of sera with different titers (in per cent); d) antihemagglutinins; e) averaged; f) neutralizing antibodies; g) undiluted; h) averaged; i) 15-20 days; j) 1-3 months; k) 4-6 months; l) 7-12 months; m) 1.5-2.5 years.

high content of neutralizing antibodies following revaccination at short intervals were not found to evidence subsequent increase in antihemagglutinin titer.

The character of skin reactions was found to be a function of the period following the last revaccination. For revaccinations after six months, positive skin reaction was noted in 23 of 103 (22.3 %), in a year -- in 51 of 132 (38.6 %), and in two years -- in 371 of 862 (43 %) of persons examined.

Comparison of the skin reaction at the original antibody level revealed that in a group of persons with negative skin reaction, the mean level of antihemagglutinins was twice as high, and the level of neutralizing antibodies even five times as high as in persons with positive skin reaction. However, significant individual variations were observed: in 5 of 23 persons with the inoculatory reaction a high antihemagglutinin level (1:40) and a high neutralizing antibody level (1:25) were recorded, while in 9 of 80 persons with negative skin reaction antihemagglutinins were not found even at a titer of 1:10. It must be noted that at a sufficiently high antibody level and positive skin reaction, further increase in antibody numbers usually was not observed. These facts evidenced that in some cases the character of skin reaction upon inoculation depended not only on the original antibody level, but also on local tissue immunity, which was supported by the following observations. When revaccinations were carried out,

TABLE 3

Antibody Level in Different Groups of Inoculants in 15-20 Days Following Last Revaccination

| (a) Интервал между прививками | (b) Число прививок | (c) Число исследованных сывороток | (d) Число сывороток с разными титрами | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (e) антигемагглютининов | | | | | | (g) нейтрализующих антител | | | | |
| | | | <1:10 | 1:10 | 1:20 | 1:40 | >1:80 | (f) в среднем | (h) неразведенная | 1:10 | 1:20 | >1:125 | (i) в среднем |
| 6 месяцев (j) | 1 | 52/38 | 17 | 20 | 11 | 7 | 1 | 1:10 | 6 | 5 | 18 | 9 | 1:25 |
| | 2 | 87/28 | 5 | 18 | 25 | 32 | 7 | 1:20 | 1 | 8 | 10 | 9 | 1:25 |
| | 3 | 58/28 | 2 | 12 | 16 | 20 | 8 | 1:20 | 2 | 6 | 14 | 6 | 1:25 |
| | 4 | 42/31 | 1 | 8 | 10 | 15 | 8 | 1:40 | 0 | 5 | 9 | 17 | >1:125 |
| | 5 | 20/15 | 2 | 6 | 5 | 4 | 3 | 1:20 | 0 | 0 | 6 | 9 | >1:125 |
| | 6 | 11/8 | 1 | 2 | 3 | 3 | 2 | 1:20 | 0 | 1 | 2 | 5 | >1:125 |
| 1 год (k) | 1 | 34/13 | 6 | 10 | 10 | 8 | 0 | 1:20 | 3 | 2 | 5 | 3 | 1:25 |
| | 2 | 13/9 | 2 | 4 | 2 | 6 | 0 | 1:20 | 0 | 0 | 3 | 6 | >1:125 |
| 2 года (l) | 1 | 32/25 | 7 | 9 | 8 | 7 | 1 | 1:15 | 8 | 9 | 7 | 1 | 1:5 |

Remarks as for Table 2.

LEGEND: a) interval between inoculations; b) number of inoculations; c) number of sera investigated; d) number of sera with titers as listed; e) antihemagglutinins; f) average; g) neutralizing antibodies; h) undiluted; i) average; j) six months; k) one year; l) two years.

a negative skin reaction was observed in 1421 inoculants. In five days, they were reinoculated with vaccine of the same series, on the other hand, and in 895 persons (62.9 %) a positive skin reaction developed.

Our observations showed that in sera containing antihemagglutinins neutralizing antibodies are always present. The number of sera containing neutralizing antibodies only in the native state was slight. However, we should recall that the absence of antihemagglutinins evidently does not point to an absence of humoral immunity, since in these persons neutralizing antibodies can be present and even sometimes at high titer.

Therefore, we do not exclude the possibility that the estimate of immunity intensity only from results of the hemagglutinin-inhibition reaction can be somewhat understated.

When revaccination was carried out at an interval of a year, the percentage of positive skin reactions exceeded 30 %, that is, the value at which mass revaccination of the region's population was necessary, where it was established through spot checks of the overall immunity. The results of serological examinations in this group of inoculants pointed to an adequately high level of humoral immunity, which confirmed the advisability of annual revaccination of specific

contingents of persons and once more showed that judgment of the loss of immunity against smallpox from an estimate of the character of skin reaction is not reliable.

Serological indices of the spot check of immunity intensity in the group of persons revaccinated at an interval of two years were satisfactory. However, it must be noted that 21.9 % of those inoculated did not have antihemagglutinins, and the number of sera neutralizing virus only in the native state amounted to 32 %. Some of these persons exhibited a low level of smallpox defense. Increasing the extent of immunity in these persons can proceed only by reducing the schedules between revaccination or by increasing the quality of the smallpox vaccines. Therefore requirements of the quality of smallpox vaccines must be raised, especially for revaccinations, up to the recommendations of the World Health Organization and control instructions of vaccine use must include the determination of preparation activities by titration on chick embryos or equivalent tests using the sensitivity of tissue cultures.

## Conclusions

1. The ratio of humoral antismallpox antibodies has been studied. Neutralizing antibodies were found in 99.1 %, antihemagglutinins -- in 86.7 %, complement-fixating antibodies -- in 50 % of sera examined. A direct relationship was found between the level of antihemagglutinins and neutralizing antibodies.

2. Following revaccination, a gradual drop in antihemagglutinin level was observed and in 1.5-2.5 years antihemagglutinins were contained in 60.25 % of the inoculants at a low titer (1:10) or were absent entirely. Neutralizing antibodies disappeared considerably more slowly and by the same period of time had been determined in 80 % of inoculants at a titer not less than 1:5.

3. The level of neutralizing antibodies depended on the total number of inoculations and intervals between such treatments. A high level of these antibodies (~~averaging 1:25 -- > 1:125~~) was observed in revaccinations performed at an interval of 6-12 months. The level of neutralizing antibodies in revaccinations made after two years was considerably lower (averaging 1:5). ~~The antihemagglutinin level~~ could not be established ~~as a clearly~~ pronounced function of the total number of inoculants and the intervals between such treatments.

4. In persons reacting negatively to inoculation, before revaccination the mean antibody level was higher than in persons reacting positively. However, significant individual fluctuations in the character of skin reaction not coinciding with the antibody level was noted.

5. The estimate of the collective immunity from results of inoculatory reactions was not reflected in the original state of immunity, therefore an appraisal of the anti-smallpox immunity is best sought for from results of sampling determination of antibodies. Revaccination of the population must be carried out in the case in which 25 % and more of sera examined do not contain antihemagglutinin at a titer of 1:10.

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