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# Humoral and Cell-Mediated Immune Responses in Humans Before and After Revaccination with Vaccinia Virus

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Twenty-six healthy males vaccinated 15 to 18 years ago with vaccinia virus were revaccinated. Blood samples were collected before vaccination and 3 weeks after. The lymphocytes were tested in a blast transformation assay with vaccinia antigen and phytohemagglutinin, and interferon production was measured. The sera were subjected to neutralization and antibody-dependent cell-mediated cytotoxicity (ADCC) tests. All results were compared with clinical responses. The only test showing immunity in all donors before vaccination was the ADCC. The other tests showed a very limited residual immunity or no immunity at all. After revaccination, immunity reactions were found in all tests in most of the donors. None of the tests made before vaccination could be used to predict clinical reactions. The ADCC is recommended as a sensitive serological test.

The mechanisms by which organisms react against and remain immune to viral infections are currently under study.

Many in vitro methods measuring different parts of immune reactions have been developed, and a variety of mechanisms involving different cell types and soluble factors have been described (9, 11). In spite of the many investigations, the mechanisms responsible for recovery from and resistance to viral infections have not been clarified. Yet it seems that cell-mediated immunity is of the greatest importance with respect to membrane-associated viruses, like poxvirus, herpesvirus, and myxovirus, whereas humoral immunity predominates in picornavirus and arbovirus (11).

The study reported below was performed to compare different parameters measuring cell-mediated and humoral immune responses to revaccination with vaccinia virus and to find out if any correlation exists between clinical manifestations and the immune parameters measured.

Blastogenesis in response to vaccinia antigen and phytohemagglutinin (PHA) and interferon production in lymphocytes stimulated with vaccinia antigen were used as parameters of cell-mediated responses, whereas neutralization tests and antibody-dependent cell-mediated cytotoxicity tests (ADCC) with antibodies in combination with foreign lymphocytes were used as parameters of humoral immune responses.

## MATERIALS AND METHODS

**Blood donors.** Healthy males doing their military service, all previously inoculated with vaccinia virus, were revaccinated and used as blood donors. Blood samples were taken on the day of revaccination and 3 weeks later.

**Revaccination.** Vaccinia virus, produced as a calf lymph virus at the State Serum Institute (SSI), Copenhagen, Denmark, was inoculated intradermally by a hollow needle at two sites on the upper arm. The vaccinations were performed by the same person to ensure a uniform vaccination technique. The skin eruptions were inspected after 1 week, and the vaccination response was recorded.

**Preparation of antigen.** Vaccinia virus SSI, batch 020176, was inoculated on HeLa cell monolayers grown in Eagle minimal essential medium with 2% calf serum. At the time of maximum cytopathic effect, the supernatant was collected and centrifuged at 2,000 × *g* for 10 min to spin down cellular debris. The supernatant, containing $4 \times 10^4$ plaque-forming units per ml, was inactivated at 56°C for 3 h and used as vaccinia antigen. Control antigen was prepared from uninfected HeLa cell cultures treated in the same way as the infected ones. Antigen pools were stored in small aliquots at −70°C.

**Preparation of leukocyte cultures for blast transformation.** Mononuclear cells were obtained from heparinized peripheral venous blood (20 IU of heparin per ml) by Ficoll-Isopaque flotation (2). The cells were washed twice in RPMI-1640 medium containing 2% heat-inactivated human serum, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid buffer, penicillin, and streptomycin. For the first wash, the medium contained 15 to 20 IU of heparin per ml. The human serum used in the medium originated from an adult male who had never received a vaccinia vaccination.

After washing, the cells were suspended in RPMI-1640 medium containing *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid buffer, penicillin, streptomycin, and 15% human serum. The cells were counted, and the suspension was diluted to $10^6$ cells

per ml and dispensed in 0.2-ml portions in the wells of a microplate (Linbro, no. IS-MRC-96). To the cultures were added 10-μl amounts of antigen, either undiluted or in dilutions of 1:4 or 1:16. PHA was added in 10-μl amounts from solutions containing 0.1 or 0.01 μg/μl. All stimulations were performed in triplicate. The plates were closed with plastic covers, and vaccinia cultures and unstimulated cultures were incubated in 5% $CO_2$ for a total of 6 days. PHA cultures were incubated for a total of 3 days.

**Thymidine incorporation.** For the final 18 h of incubation, 1 μl of [2-$^{14}$C]thymidine solution (0.02 μCi/μl) was added to each well. The cells were harvested with a Skatron harvester, type POB 8, which delivers the washes on glass-fiber paper (Whatman GF 81). The paper disks were placed in plastic counting vials with 2 ml of triton toluol scintillation fluid, and the counts were recorded on a Packard Tri-Carb liquid scintillation spectrometer. The counts per minute were converted to disintegrations per minute. The results are given both as incremental counts (vaccinia stimulated cultures minus control stimulated cultures) and as stimulation indexes (vaccinia stimulated cultures/control stimulated cultures). The results from PHA cultures and unstimulated cultures are given in disintegrations per minute.

**Interferon assay.** The supernatant from vaccinia antigen-stimulated lymphocytes (10 μl/2 × $10^5$ cells in 0.2 ml) was harvested after incubation for 6 days and centrifuged at 3,000 × *g* for 10 min. The assay was done using a micromethod by which MicroTest II (Falcon) wells were seeded with 2.5 × $10^4$ human embryonic skin fibroblasts in a 0.1-ml volume using Eagle minimal essential medium with 10% calf serum. After growth to confluence, 0.05 ml of the supernatant to be tested was added to wells in the first row. Further threefold dilutions were made directly in the wells with an automatic microdiluter equipped with 0.05-ml diluting loops. All samples were tested in duplicate rows (6). An interferon standard of known titer was included on each plate. The cells were challenged with vesicular stomatitis virus after 18 h, and cytopatic effect was read microscopically after incubation for 24 h. The interferon titer is the dilution that reduced vesicular stomatitis virus cytopathic effect by 50%.

**Characterization of interferons.** Interferons were characterized as type I (classical) or type II (immune) interferons according to the description by Valle et al. (15); i.e., influence of exposure to 56°C for 1 h, influence of exposure to pH 2 for 24 h, and neutralization with anti-human leukocyte interferon sera.

**Neutralization test.** All sera were diluted 1:2, 1:8, 1:16, and 1:32 and mixed with equal volumes of virus suspension containing 50 to 100 plaque-forming units of our vaccinia vaccination strain. The mixture was incubated for 1 h at 37°C. The test was performed as a plaque neutralization assay on human embryonic lung fibroblasts with a methylcellulose overlay. Plaque reduction of 50% was taken as the end point, and a known positive serum and a known negative serum were included in all the neutralization tests. Sera were collected before vaccination and 3 weeks later.

**Preparation of target cells for cytotoxicity assay.** Human fibroblasts originating from skin biopsies from healthy human adults, obtained as described by Therkelsen (14), were grown in monolayers in 250-ml Falcon bottles. Two to three days after being passaged, the cells were infected with vaccinia at 0.1 plaque-forming units per cell in 5 ml of medium. After 24 h, about one-third of the cells showed a well-developed cytopathic effect. The cells were trypsinized, washed, and suspended in tissue culture medium TC-199 with 5% heat-inactivated fetal calf serum. Bottles with uninfected cells were treated in the same manner.

**Preparation of lymphocytes for cytotoxicity assay.** Buffy-coat cells from unknown donors were used as effector cells in the ADCC.

As described in previous papers on ADCC against target cells infected with herpes simplex virus type 1 (7, 10), lymphocytes from one buffy coat (2 × $10^8$ to 6 × $10^8$ cells) washed seven times in TC-199 with 5% fetal calf serum were used as effector cells.

**Sera used in cytotoxicity assay.** Serum samples collected before and 3 weeks after vaccination were inactivated and assayed in ADCC in three concentrations, undiluted and diluted to 1:$10^2$ and 1:$10^4$. Two human adult sera, one with antibodies against vaccinia and one from a person never vaccinated, were used as standard control sera.

**ADCC assay.** The lymphocyte suspension was adjusted to contain $10^6$ cells per ml. Portions of 1 ml from this suspension were placed in conical plastic tubes (Nunc Plastic, 11 by 70 mm), and 60 μl of serum or serum dilution was added.

Fibroblasts to be used as target cells (infected and control cells) were labeled with $^{51}$Cr according to standard methods and added in 0.1-ml volumes from a cell suspension containing $10^5$ cells per ml. Total activity and spontaneous release were determined from tubes containing medium with 5% fetal calf serum or 60 μl of human serum and target cells without addition of lymphocytes.

Test tubes were capped and incubated for 16 to 18 h. Then, after thorough agitation followed by centrifugation at 200 × *g* for 10 min, 0.7 ml of the supernatant was withdrawn for determination of $^{51}$Cr release.

Test tubes with infected target cells were run in parallel, whereas tubes containing control target cells mostly were single, as the counts from control tubes usually were at the same level.

Specific $^{51}$Cr release was calculated by subtracting the percentage of $^{51}$Cr release of the control target cells from the mean percentage of $^{51}$Cr release of the infected cells. The percentage of $^{51}$Cr release was calculated according to the following formula: $[(A-B) \times 100]/(C-B)$ = percentage of $^{51}$Cr release, where *A* is release from target, serum, and lymphocytes, *B* is spontaneous release, and *C* is total activity in the supernatant and pellet (corresponding in volume to A and B).

## RESULTS

**Skin eruptions.** As seen from Table 1, only five donors showed an accelerated (major) reaction, which is seen in individuals with a limited degree of residual immunity from previous vaccination, whereas the rest showed an immediate

TABLE 1. *Clinical response to vaccination and influence on neutralizing antibodies in serum*

| Donor no. | Clinical response (reaction) | Neutralizing antibody titer | |
|---|---|---|---|
| | | Prevaccination | 3 weeks postvaccination |
| 2210 | Immune | —[a] | 16 |
| 2211 | Immune | — | 16 |
| 2213 | Immune | — | — |
| 2214 | Immune | — | 16 |
| 2215 | Immune | — | 4 |
| 2216 | Major | — | 4 |
| 2217 | Immune | — | 4 |
| 2218 | Immune | — | 4 |
| 2219 | Immune | — | 16 |
| 2220 | Immune | — | 32 |
| 2221 | Major | — | 16 |
| 2222 | Immune | — | 16 |
| 2223 | Immune | — | 4 |
| 2224 | Major | — | 16 |
| 2225 | Immune | — | 16 |
| 2226 | Immune | — | 16 |
| 2227 | Immune | — | 16 |
| 2228 | Major | 4 | 32 |
| 2229 | Major | — | 16 |
| 2230 | Immune | 4 | 4 |
| 2231 | Immune | — | 32 |
| 2232 | Immune | 4 | 16 |
| 2233 | Immune | — | 4 |
| 2234 | Immune | 4 | 16 |
| 2235 | Immune | 4 | 16 |
| 2236 | Immune | 4 | 4 |

[a] —, Negative in 1:4 dilution.

(immune) reaction, which is usually seen in immune individuals.

**Effect of revaccination on blast transformation.** Initial experiments with different antigen preparations were performed. During these experiments, using previously vaccinated healthy human adults as lymphocyte donors, we found only one person, revaccinated 9 months previously, who responded properly to the antigen preparations. The others, vaccinated or revaccinated more than 2 years previously, did not respond.

In agreement with this, there was no or only a slight stimulation of lymphocytes obtained before vaccination, whereas lymphocytes obtained 3 weeks after vaccination were stimulated by the vaccinia antigen to incorporate [$^{14}$C]thymidine (Fig. 1). Only two donors did not respond after revaccination (stimulation index $\leq 2$). PHA cultures were made to ensure that the lymphocytes could be stimulated. As seen from Table 2, all donors were stimulated by PHA. All counts shown for PHA cultures are from the highest concentration used. Counts from vaccinia-stimulated cultures and from control antigen-stimulated cultures given in Table 2 are from the dilution resulting in the highest counts, which differed from donor to donor.

The stimulation indexes from individuals with major reactions showed a mean value (plus or minus standard error) of 22.4 ± 5.9, and only one showed a stimulation index below 18. The mean value for individuals with immune reactions was 10.7 ± 2.1, but also in this group some with a high stimulation index were seen.

**Interferon production.** As seen from Table 2, there was no interferon production in the cultures of leukocytes obtained before revaccination, whereas 14 individuals showed interferon production after revaccination.

Comparing skin eruptions, individuals with major reactions had a mean titer of 7.8 ± 1.2, whereas the others had a mean of 3.0 ± 0.7. Interferon values of <3 were set equal to 1. The interferons produced were characterized as type 1 interferon, i.e., classical interferon.

**Neutralization test.** Only six pre-revaccination sera showed neutralizing activity against vaccinia (Table 1); the rest were found with neutralizing titers lower than 1:4. After revaccination, one donor (2213) was still without neutralizing antibodies, and one (2236) showed the same titer as before vaccination (both donors showed a higher incorporation of [$^{14}$C]thymidine in the blast transformation assay, indicating influence of revaccination on the immune response), whereas the other donors showed higher titers of neutralizing antibodies.

**ADCC.** Preliminary experiments were performed to find the proper infective dose and the proper length of infection to make acutely infected target cells well suited for the cytotoxicity

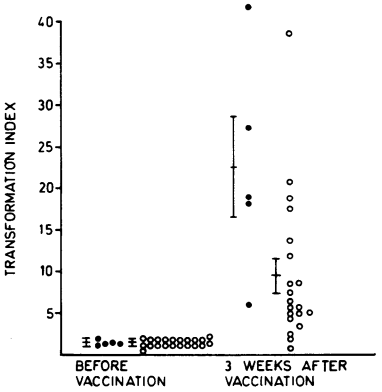


FIG. 1. *Comparison of transformation indexes in lymphocytes from donors with major reactions* (●) *and from donors with immune reactions* (○) *before and 3 weeks after revaccination with vaccinia virus.* (‡) *Mean plus or minus standard error.*

TABLE 2. *Effect of vaccination on blast transformation and interferon production*

| Donor no. | Incremental counts (vaccinia–control [dpm]) | | Stimulation index (vaccinia/control) | | PHA culture (dpm) | | Lymphocytes alone (dpm) | | Interferon titer | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5/25[a] | 6/16[a] | 5/25[a] | 6/16[a] | 5/25[a] | 6/16[a] | 5/25[a] | 6/16[a] | 5/25[a] | 6/16[a] |
| 2210 | 75 | 931 | 1.3 | 13.8 | 7,712 | 8,728 | 300 | 73 | <3 | <3 |
| 2211 | −89 | 2,130 | 0.8 | 6.5 | 4,455 | 12,225 | 380 | 77 | <3 | 9 |
| 2213 | 293 | 531 | 1.6 | 5.0 | 4,981 | 6,510 | 429 | 82 | <3 | <3 |
| 2214 | 131 | 568 | 1.2 | 5.0 | 5,796 | 9,744 | 608 | 235 | <3 | 3 |
| 2215 | 77 | 44 | 1.2 | 1.1 | 6,388 | 9,815 | 421 | 504 | <3 | <3 |
| 2216[b] | −128 | 4,838 | 0.8 | 18.1 | 6,210 | 7,329 | —[c] | 91 | <3 | 9 |
| 2217 | −391 | 1,737 | 0.6 | 11.9 | 7,615 | 9,966 | 499 | 120 | <3 | <3 |
| 2218 | 101 | 484 | 1.2 | 2.6 | 8,081 | 8,080 | 530 | 75 | <3 | <3 |
| 2219 | 177 | 4,021 | 1.1 | 38.6 | 7,253 | 3,800 | 1,254 | 106 | <3 | 3 |
| 2220 | 541 | 2,476 | 1.4 | 17.6 | 7,225 | 9,099 | 1,721 | 75 | <3 | 3 |
| 2221[b] | 639 | 3,020 | 1.9 | 41.8 | 6,605 | 9,776 | 657 | 95 | <3 | 9 |
| 2222 | 274 | 704 | 1.7 | 5.6 | 5,990 | 8,592 | 426 | 233 | <3 | <3 |
| 2223 | 279 | 158 | 1.5 | 5.0 | 7,790 | 8,325 | 644 | 34 | <3 | <3 |
| 2224[b] | 20 | 1,233 | 1.1 | 6.0 | 4,667 | 5,962 | 247 | 161 | <3 | 9 |
| 2225 | 394 | 2,490 | 1.7 | 8.8 | 4,680 | 7,188 | 1,069 | 129 | <3 | 9 |
| 2226 | 95 | 441 | 1.2 | 4.6 | 6,612 | 11,735 | 391 | 583 | <3 | 3 |
| 2227 | 50 | 2,544 | 1.3 | 18.9 | 4,333 | 6,937 | 240 | 92 | <3 | <3 |
| 2228[b] | 111 | 4,742 | 1.5 | 27.3 | 6,142 | 7,442 | 280 | 127 | <3 | 9 |
| 2229[b] | 74 | 1,130 | 1.3 | 18.9 | 5,781 | 10,538 | 163 | 46 | <3 | 3 |
| 2230 | 308 | 2,135 | 1.9 | 5.7 | 5,159 | 9,610 | 532 | 339 | <3 | 9 |
| 2231 | 94 | 1,477 | 1.4 | 7.5 | 3,122 | 8,691 | 285 | 540 | <3 | 3 |
| 2232 | 317 | 1,241 | 2.3 | 8.6 | 6,016 | 4,485 | 417 | 158 | <3 | 9 |
| 2233 | — | 1,743 | — | 31.1 | — | 11,191 | — | 60 | <3 | <3 |
| 2234 | 140 | 62 | 1.3 | 2.0 | 4,321 | 7,061 | 647 | 61 | <3 | <3 |
| 2235 | 196 | 183 | 1.4 | 3.3 | 5,329 | 8,516 | 412 | 71 | <3 | <3 |
| 2236 | 258 | 3,349 | 1.8 | 20.7 | 5,654 | 8,037 | 360 | 189 | <3 | <3 |

[a] Dates of sample collection: 5/25, prevaccination; 6/16, 3 weeks postvaccination.
[b] Donor with major reaction.
[c] —, Not done.

assay. In addition, investigations were made to find out if the technique using extensively washed effector cells from an unknown donor, as in a previous study with herpes simplex virus-infected target cells, could be used in vaccinia-infected target cells. Also in this system, the ADCC proved to be a very sensitive serological test, and, in accordance with this, all previously vaccinated individuals showed antivaccinia activity in their serum, whereas one adult and three children who had never been vaccinated showed no activity (Table 3).

When used to test undiluted serum from our donors, the percentages of killing ranged from 6.7 to 26.9 with sera obtained before revaccination (Table 4) and from 10.1 to 32.5 after revaccination.

In sera from before revaccination, no differences were found in the ADCC between donors showing a major reaction and those with an immune reaction. After revaccination, a nonsignificantly higher ADCC activity was found in sera from donors with a major reaction (26.1 ± 1.5 versus 21.4 ± 1.2). Table 4 also shows the results from sera diluted to 1:100, in which cytotoxicity could still be found, whereas no cytotoxic activity could be demonstrated in dilutions of 1:10,000. Serum from a control donor who had never been vaccinated invariably showed a percentage of killing below 1.5.

TABLE 3. *Percentage of killing of vaccinia-infected fibroblasts in an ADCC system using the same effector lymphocytes (buffy coat) with different sera*

| Serum donor (age) | Specific $^{51}Cr$ release (%) |
|---|---|
| Previously vaccinated | |
| J.Å. | 20.6 |
| Fl.R. | 16.1 |
| V.S. | 24.6 |
| L.L. | 15.8 |
| F.H. | 17.0 |
| K.B. | 21.3 |
| A.M.L. | 21.1 |
| Never vaccinated | |
| I.J. | 1.4 |
| L.S. (2 yr) | 1.3 |
| B.N. (1 yr) | 0.7 |
| L.N. (1 yr) | 1.3 |
| Fetal calf serum | 2.3 |

TABLE 4. *Percentages of killing of vaccinia-infected fibroblasts in an ADCC system, using serum collected from donors before (5/26) and after (6/16) revaccination with vaccinia virus. The same effector lymphocytes (buffy coat) were used*

| Donor no. | Specific $^{51}Cr$ release (%) | | | |
|---|---|---|---|---|
| | 5/26 serum | | 6/16 serum | |
| | Undiluted | 1:$10^2$ | Undiluted | 1:$10^2$ |
| 2210 | 7.5 | 1.7 | 11.9 | 3.0 |
| 2211 | 6.7 | 0.1 | 21.7 | 13.2 |
| 2213 | 14.6 | 4.5 | 16.5 | 3.9 |
| 2214 | 10.6 | 5.8 | 32.5 | 15.9 |
| 2215 | 18.1 | 0.6 | 23.3 | 6.9 |
| 2216 | 10.4 | 2.1 | 29.8 | 16.1 |
| 2217 | 17.5 | 4.5 | 19.8 | 3.4 |
| 2218 | 11.0 | 3.5 | 10.1 | 1.7 |
| 2219 | 22.2 | 4.0 | 20.1 | 10.0 |
| 2220 | 11.4 | 0.9 | 19.5 | 6.7 |
| 2221 | 14.4 | 1.4 | 26.7 | 10.5 |
| 2222 | 7.0 | −1.1 | 19.2 | −0.3 |
| 2223 | 16.7 | 3.8 | 24.1 | 3.9 |
| 2224 | 10.3 | −3.2 | 21.1 | 4.7 |
| 2225 | 7.1 | −2.8 | 19.9 | 2.9 |
| 2226 | 13.3 | 2.1 | 30.7 | 4.4 |
| 2227 | 21.0 | 1.7 | 19.9 | 4.4 |
| 2228 | 18.8 | 2.9 | 30.3 | 13.4 |
| 2229 | 7.0 | 0.3 | 22.2 | 4.0 |
| 2230 | 15.2 | −0.9 | 11.8 | −1.6 |
| 2231 | 23.0 | 3.4 | 23.8 | 8.0 |
| 2232 | 14.2 | 3.3 | 22.6 | 5.5 |
| 2233 | 26.9 | 6.9 | 27.6 | 14.2 |
| 2234 | 15.0 | 2.6 | 26.0 | 7.7 |
| 2235 | 14.9 | 4.9 | 23.3 | 3.5 |
| 2236 | 23.7 | 4.4 | 24.1 | 6.2 |
| Negative control | ≤1.5 | | ≤1.5 | |

## DISCUSSION

Although "natural" infections with smallpox seem to be eradicated and with them, the use of vaccinia vaccinations, we consider this vaccination a type of "infection" well suited for laboratory investigations of immune reactions, since it shares properties with other viral infections.

In this study, we tested parameters of both humoral and cellular immunity to analyze residual immunity after previous vaccination, most often done 15 to 18 years previously, and to follow the developments in these parameters after vaccination. We also wanted to discover if any correlation could be found between the parameters studied and clinical response after vaccination.

The clinical response after vaccination could clearly be divided into major and immune reactions. Unfortunately, there was an uneven distribution between the two groups, with only five donors showing a major reaction. In spite of this small number, we have compared the groups. The major reaction is supposed to indicate virus multiplication, and persons in whom this reaction occurs are considered to possess only a limited degree of residual immunity. The immune reaction may represent immunity, but it may also represent merely an allergic reaction of the delayed-hypersensitivity type. The latter can be seen after vaccination with inactivated virus or if an inadequate technique is used (4). As all our donors with immune reactions showed responses in one or more of the parameters measured here, we consider the vaccine and the technique adequate, and we consider the immune reactions obtained in our study to be an expression of a higher degree of immunity than that in the group with major reactions. Although none of the parameters could be used to predict clinical reactions, as the prevaccination results were at the same level in the two groups, several of the tests showed a higher response in donors having a major reaction, indicating a more intensive stimulation of immunity in these cases. In blast transformation, we found low levels of stimulation before vaccination, although only three donors showed indexes below 1. As inactivated vaccinia is reported to give indexes below 1 in negative individuals (8, 13), values above 1 might reflect a slight degree of cell-mediated immunity before vaccination. Revaccination resulted in a considerable reaction against vaccinia antigen. Blast transformation with vaccinia antigen has given conflicting results. Using inactivated virus and human lymphocyte-macrophage cultures, Epstein et al. (5) found high stimulation before revaccination, whereas, in rabbits, Rosenberg et al. (13) observed high levels of stimulation of lymphocytes with vaccinia antigen only during acute infections. Variations in cultures and antigens could explain these differences.

The interferon produced by stimulating Ficoll-Isopaque cells with inactivated vaccinia antigen was always characterized as type 1 (classical) interferon. This is in agreement with a previous study by Haahr et al. (6), who used herpes simplex virus as antigen. Interferon was never produced in lymphocytes before revaccination, whereas most donors showed interferon production after vaccination, which was at slightly higher levels in donors with major reactions.

The major reaction was found to occur at random in donors with and without neutralizing antibodies. The rise in neutralizing antibodies was roughly the same in donors showing a major reaction and those with an immune reaction.

The ADCC was the only immunological test

showing consistent immunity against vaccinia before revaccination. As shown in the herpes system, (10) this test is very well suited to measure residual immunity in cases where this cannot be detected by other serological tests. It might be presumed that this might also apply to other virus systems, and difficulties in cytotoxicity tests, where "seronegatives" show positive killing (reported especially by two groups working with mumps [1, 3]), might be due to small amounts of antibodies acting in an ADCC system. Furthermore, the ADCC is probably an in vitro test comparable to the in vivo situation, as mice made extremely susceptible to herpes simplex virus type 1 infection by a dose of cyclophosphamide could be protected by the administration of immune serum plus normal spleen cells (12).

In this investigation, we have only used extensively washed lymphocytes from an unknown donor as effector cells in the ADCC. We consider it important to find out how lymphocytes from revaccinated donors behave in a cytotoxicity system without antibodies during the first few weeks after vaccination; the activity during this period might be due to T-lymphocytes. Such an investigation is in progress in our laboratory.

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