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Cell-Mediated Immune Responses to Smallpox Vaccination

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We report that vaccine dilution (1:1 or 1:10) and previous vaccinia virus vaccination status had no significant effect on cell-mediated immune responses (i.e., the immediate vaccinia virus-specific gamma interferon-producing T-cell response measured by enzyme-linked immunospot assay) 1 month after smallpox vaccination (Lancy-Vaxina; Berna Biotech, Switzerland).

The evaluation of successful vaccination with vaccinia virus is based on visual confirmation of a dermal response (10). However, assessment of the effectiveness of the attenuated forms of smallpox vaccines (i.e., modified vaccinia Ankara strain, New York City Board of Health strain [NYCBH], or LC16m8) and the next generation of smallpox vaccines requires a broader understanding of the immune response to vaccinia virus (4). However, there are limited and sometimes conflicting data on the dependence of human cell-mediated immune responses to smallpox vaccination on vaccine dilutions (3, 5, 9) and previous vaccinia vaccination status (4, 5, 6). We have therefore evaluated whether vaccine dilutions or previous vaccination status affect the induction of cell-mediated immune responses to smallpox vaccination.

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A randomized single-blind controlled trial comparing the efficacies of undiluted (1:1) and diluted (1:10) Lancy-Vaxina vaccine (Berna Biotech, Switzerland) was conducted at Seoul National University Hospital between February 2003 and October in 2004 (8). Lancy-Vaxina smallpox vaccine was derived from the Lister/Elstree strain.

Cell-mediated immune responses to smallpox vaccination were assessed as the immediate vaccinia virus-specific gamma interferon (IFN-γ)-producing T-cell response by enzyme-linked immunospot (ELISPOT) assays. The assay was performed as described previously (1, 7). Briefly, approximately 60 ml of venous blood was obtained from each volunteer in this trial just before vaccination (day 0) and 30 days after vaccination. Within 6 h of collection, peripheral blood mononuclear cells (PBMC) were isolated by using Ficoll-Hypaque density gradients. The PBMC were then resuspended at a concentration of 10^7 cells/ml in RPMI 1640–20% fetal bovine serum–10% dimethyl sulfoxide and were cryopreserved. The cryopreserved PBMC were thawed and washed once with RPMI 1640 supplemented with 10% fetal bovine serum and 50 U of Benzonase (Sigma-Aldrich)/ml. Cells were again washed and then resuspended with RPMI 1640 supplemented with 10% fetal bovine serum at a concentration of 5×10^6 cells/ml. The prepared PBMC were infected for 1 h with the live vaccinia virus (fresh vaccinia virus obtained from lyophilized Lancy-Vaxina vaccine) at a multiplicity of infection of 1. Cells were washed and added to 96-well ELISPOT plates (BD Biosciences Pharmingen) coated with anti-human IFN-γ antibody (BD ELISPOT human IFN-γ kit). Negative controls were uninfected cells with medium alone. Positive controls were uninfected PBMC stimulated with purified phytohemagglutinin (Sigma-Aldrich). Cells were cultured in duplicate wells at 5×10^5 cells/well at 37°C for 18 h. Spots were counted by use of an automated microscope (Carl Zeiss MicroImaging, Inc., Germany) after the background value, obtained by using unstimulated cells, was subtracted. A postvaccination response for cell-mediated immunity was defined as positive if the vaccinia virus-specific IFN-γ-producing T-cell response by ELISPOT assays increased by two times or more compared to prevaccination and >20 spot-forming cells (SFC)/10^6 PBMC (after subtracting the background obtained with unstimulated cells) (4). This threshold was established by taking into account the SFC range in the 16 vaccinia virus-naive individuals used as negative controls (median = 1.2 SFC/10^6 PBMC [range = 0 to 19 SFC/10^6 PBMC]).

Fifty-five paired PBMC were obtained from the 112 subjects who had "take" reactions. The 55 participants included 16 vaccinia virus-naive and 39 previously vaccinated persons who had been vaccinated before 1978. Nineteen received undiluted vaccine, and 36 received a 1:10 dilution. The mean age (± the standard deviation) of the subjects was 32.1 (±7.6) years, 38 (69%) of whom were male. Of the 55 participants, 42 (76%) gave a positive postvaccination response for cell-mediated immunity. Fourteen (74%) of the nineteen who received undiluted vaccine and twenty-eight (78%) of the thirty-six who received the 1:10 dilution had positive postvaccination cell-mediated immune responses ($P = 0.75$). Of the 16 vaccinia virus-naive persons (2 receiving undiluted vaccine and 14 receiving the 1:10 dilution), 12 (75%) had positive postvaccination cell-mediated immune responses, and 30 (77%) of the 39 previously vaccinated persons (17 received undiluted vaccine, and 22 received the 1:10 dilution) had positive postvaccination cell-mediated immune responses ($P = 0.99$). A scatter plot

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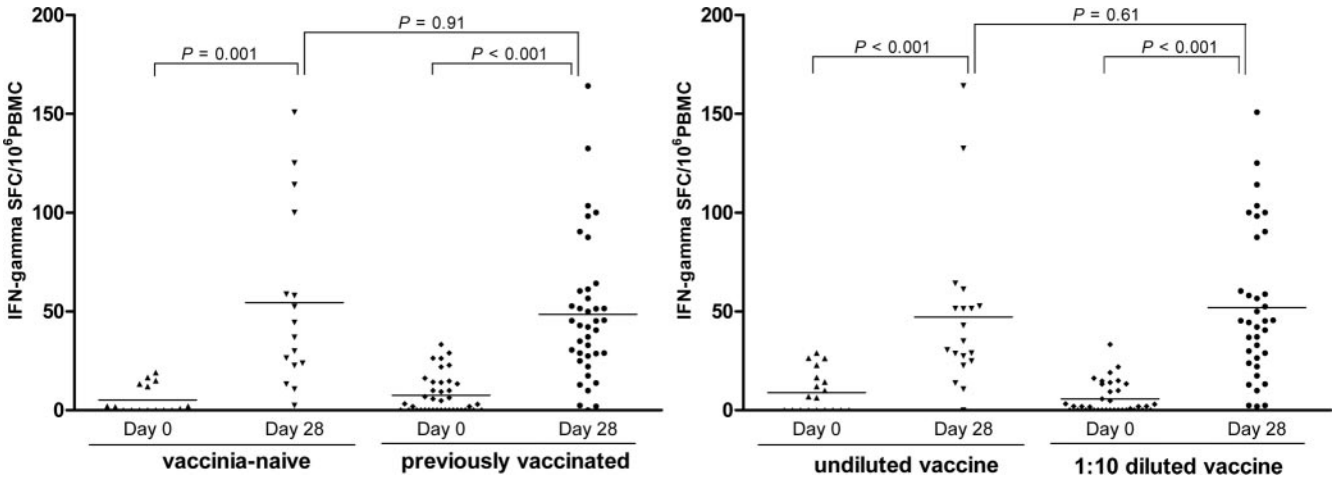

FIG. 1. Dot plot showing the distributions of the immediate vaccinia virus-specific IFN-γ-producing T-cell responses before and 1 month after smallpox vaccination with respect to the vaccine dilutions and previous vaccination status. The bars indicate the means. A Mann-Whitney U test was used to compare the two groups.

giving the actual data obtained with the ELISPOT assay is presented in Fig. 1.

Immune responses to smallpox vaccinations have been analyzed almost exclusively by measuring antibodies in serum samples. Therefore, data on cytotoxic T-cell responses after smallpox vaccination are limited (2, 10, 11), especially in relation to vaccine dilutions (3, 5, 9) and preexisting immunity to vaccinia virus (4, 5, 6). Frey et al. reported that 1:10-diluted smallpox vaccine ($n = 18$) resulted in significantly fewer cell-mediated immune responses 6 months after vaccination than did undiluted vaccine ($n = 20$) (3). However, Hsieh et al. found that vaccine dilution did not affect cell-mediated immune responses 1 month after smallpox vaccination (5). Rock et al. recently demonstrated that 1 month after smallpox vaccination, $CD4^+$ and $CD8^+$ T-cell responses in subjects who received diluted Aventis Pasteur smallpox vaccine were similar to those in subjects who received undiluted vaccine (9). Our finding of no significant difference between undiluted and 1:10-diluted vaccine in cell-mediated immune responses 1 month after smallpox vaccination is consistent with the latter two studies (5, 9). Possible explanations for the different results of Frey et al. (3) could be the differences in vaccine strain (NYCBH strain versus Lister strain), vaccine dose ($10^{6.5}$ PFU/ml [3] versus $10^{6.7}$ PFU/ml [the present study] and $10^{7.5}$ PFU/ml [5], or the assay methods for cell-mediated immunity (ELISPOT assay [3; the present study]) versus the intracellular cytokine stain assay (9) and vaccinia virus-specific CD69 expression (5).

Only a few reports have addressed the impact of previous vaccination status on cell-mediated immune responses after smallpox vaccination. Kennedy et al. reported that previously vaccinated persons ($n = 19$) developed a more rapid cell-mediated immune response at 1 or 2 weeks after smallpox vaccination than vaccine-naive persons ($n = 10$) but that the two groups had similar levels of cell-mediated immune responses 1 month after vaccination (6). Hsieh et al. also found that previous vaccination status did not affect the cell-mediated immune response 1 month after smallpox vaccination (5), and Greenberg et al. obtained the same result using a new cell-cultured smallpox vaccine (4). Our finding on the lack of effect of previous vaccination status is thus in line with these previous studies (4, 5, 6). In an earlier report (8), we also showed that a certain extent of vaccine dilution, and preexisting immunity to vaccinia virus, did not affect the immunologic set points of the humoral immune response 1 month after smallpox vaccination. We thus assume that once viral replication is initiated in the skin, the ensuing humoral and cellular immune responses are similar, regardless of the dose of vaccine or immune status.

In the present study, only 42 (76%) of 55 subjects who had "take" reactions and showed positive humoral immune responses gave positive responses by ELISPOT assay 1 month after Lancy-Vaxina vaccination. Comparison with a previous report that 31 (91%) of 34 subjects had positive cell-mediated immune responses 6 months after Dryvax vaccination (3) might suggest that the cell-mediated immune response assessed by our ELISPOT assay may have been suboptimal. Furthermore, the level of vaccinia virus-specific T cells (i.e., the mean was 50 SFC/10^6 PBMC) was lower than in previous reports (2, 3, 11). However, our result was similar to that of Kennedy et al., who determined a geometric mean of 56 SFC/10^6 PBMC in persons with the "take" reaction on day 28 after smallpox vaccination (6). Possible explanations for the observed differences could be differences in in vitro stimulation techniques. Peripheral blood monocytes were used as antigen-presenting cells in the present study and some others (1, 10), whereas autologous Epstein-Barr virus-transformed cells were used in other investigations (2, 3, 6, 11). In addition, we used PBMC stimulated with vaccinia virus for a short time (1 h) as in a previous study (1), while longer stimulation times (more than 18 h) were used by others (9, 10). Differences in the virus strain used for vaccination could also affect the result as mentioned above; the Lister strain (the present study) versus the NYCBH strain (2, 3, 4, 6, 9, 10, 11). In addition, the IFN-γ-producing spots in the ELISPOT assay do not measure $CD8^+$ cytotoxic T cells directly since other IFN-γ-producing cells, such as NK cells or noncytotoxic cells, also contribute to the IFN-γ-producing spots. However, Ennis et al. (2) reported that "the vaccinia virus-specific IFN-γ-producing T cells were predominantly $CD8^+$ after stimulation of PBMC with live vaccinia

virus." Hence, we did not pursue a detailed analysis of the IFN-γ-producing cells in the present study.

In conclusion, our data suggest that vaccine dilution and previous vaccination status do not affect the set points of the cell-mediated immune response 1 month after smallpox vaccination in persons with "take" reactions.

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