Serum Neutralizing Antibody and Lymphocyte Transformation Responses After Influenza B Virus Infections

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Serum neutralizing antibody and influenza B-specific lymphocyte blast transformation responses were measured in 110 adults and children after an influenza B outbreak. Serum neutralizing antibody and lymphocyte blast transformation responses were seen in 67 to 75% of adults and children recently infected (<1 year), but significant lymphocyte blast transformation responses were seen in only 25% of those whose infection was remote (≥3 years). The frequencies of influenza B-induced lymphocyte blast transformation and serum neutralizing antibody responses were similar in the adults and children with similar infection histories.

Influenza virus infection and vaccination induces both humoral and cell-mediated immune responses in adults and children (3, 5, 8, 9, 13, 14). Studies of cell-mediated immune responses in children have been limited to responses induced by influenza A antigens (7, 13). The occurrence of an influenza B epidemic in the season of 1979 to 1980 among families in the Houston Family Study (12) offered an opportunity to evaluate immune responses to virus infection in children and adults.

A subgroup of the Houston Family Study (39 families with 72 children aged 1 to 16 years and 38 adults) was selected because appropriate clinical specimens for the influenza season of 1979 to 1980 were available. All family members were observed virologically and serologically for occurrence of virus infection throughout the year. Infections were classified as primary in children enrolled in the family study at birth if they had no prior diagnostic evidence of influenza B infection. An infection in an adult was classified as primary if the individual had no preexisting serum neutralizing (Nt) antibody (Ab) (<1 log₂) to the virus of the 1979 to 1980 epidemic (B/Singapore/222/75) and no laboratory-confirmed influenza B virus infection since joining the family study (1975 through 1978). Reinfection was defined by virus isolation or a rise in Ab titer in an individual previously identified as infected with a type B influenza virus.

A serum sample was obtained from each study participant in the fall of 1979. A heparinized blood specimen was collected after the influenza B epidemic in the spring of 1980 at intervals ranging from 2 to 12 weeks postinfection. Third blood samples were obtained in August and October 1980 to identify occurrences of additional serological rises. Lymphocytes obtained by Ficoll-Hypaque gradient separation (2) were suspended to 10⁶ cells per ml in RPMI-1640 medium supplemented with glutamine and antibiotics (GIBCO Laboratories, Grand Island, N.Y.).

A Formalin-inactivated monovalent influenza B/Hong Kong/72 whole virus vaccine (Merck Sharp & Dohme, West Point, Pa.) after dialysis against phosphate-buffered saline was diluted in RPMI-1640 for use in the lymphocyte blast transformation (LBT) assay at concentrations indicated as optimal by preliminary studies. The antigen and cell controls consisted of chorioallantoic fluid and RPMI-1640, respectively. Mitogen stimulation by phytohemagglutinin (Burroughs-Wellcome Co., Research Triangle Park, N.C.) was done to demonstrate the capacity of lymphocytes to undergo transformation.

LBT assays were performed in microtiter plates containing 10⁵ lymphocytes per well with 0.1 ml of 400, 80, or 40 hemagglutination units of B/Hong Kong/72 virus vaccine, chorioallantoic fluid, or RPMI added in triplicate wells. After 4 days of incubation, 1 μCi of [³H]thymidine was added to each well, and the cells were harvested 24 h later. Results were expressed as a stimulation index which was calculated by dividing the mean counts per minute of B/Hong Kong/72 virus-stimulated cells by the mean counts per minute of control antigen-stimulated cells. Significant stimulation values were considered to be ≥3.0.

Nasal wash specimens and throat swabs collected during illnesses were tested for virus as previously described (1, 10). Microneutraliza-
tion Ab assays (11) were performed with the B/Singapore virus on all serum samples obtained in 1976 to 1977 and 1979 to 1980.

Serological or virological evidence of infection with influenza B virus was detected in 32 children and 15 adults from 19 families. Twenty-two children and nine adults shed virus, and serum Ab rises occurred in all of these except three young children. The frequencies of LBT responses after infection were similar in both age groups; LBT responses occurred in 24 of 32 (75%) children and 10 of 15 (67%) adults.

Primary influenza B virus infection (Fig. 1A) was detected in 24 of 43 (56%) children (19 were <6 years old) and 4 of 6 adults. Nineteen children had infection proven by virus isolation (15 were <6 years old), and 5 exhibited only a serological rise. An LBT response was seen in all children except for four who shed virus and one with an Ab rise. Postinfection serum Nt Ab titers ranged from ≤1 to 4.5 log; for both children and adults. No correlation was noted between the Nt Ab titer and LBT response in children (r = 0.18, P > 0.4; Spearman rank test). Four of five children without an LBT response had virologically proven infections with influenza B virus, and three had no measurable Nt Ab response. In four children with an LBT response 4 to 8 weeks after an isolate-proven infection, a Nt Ab rise was not demonstrable until the second follow-up specimen was examined.

Reinfection during the 1979 to 1980 influenza B outbreak occurred in 8 children (7 of whom were ≥8 years of age) and 11 adults. As shown in Fig. 1B, there was no significant difference in the frequency of LBT responses in reinjected adults and children (0 of 11 versus 5 of 8; P > 0.05, Fisher exact test), although the geometric mean Nt Ab titer was significantly higher in the latter group. Correlation between Ab titer and LBT response could not be demonstrated in either age group, even when the four adults who were classified as having primary infections were considered to have been previously primed with influenza B virus and were included in the reinfection group (r = 0.6, P > 0.4 and r = 0.4, P > 0.1, respectively).

The frequency of LBT responses was also similar in children and adults (7 of 21 versus 6 of 22) who had serological evidence of a prior influenza B virus infection and who showed no evidence of being reinjected during the 1979 to 1980 season (Fig. 1C). An LBT response was more likely to be seen in the children after a recent primary influenza B virus infection (Fig. 1A) than if they had had only a remote infection (Fig. 1C) (19 of 24 versus 7 of 21; P < 0.005, Fisher exact test). No such difference could be detected in the few adults defined as having a recent primary influenza B virus infection (1 of 4 versus 6 of 22; P > 0.05). Again, no correlation was seen between the Nt Ab titer and LBT response in the children or the adults with remote infections (r = 0.04, P > 0.8; r = 0.11, P > 0.6).

An LBT response was seen in only 1 of 19 children and 1 of 2 adults who were Nt Ab negative and who lacked virological evidence of
an influenza B virus infection (data not shown). These studies indicate that lymphocytes from children and adults with primary influenza B virus infections or with reinfections were able to recognize a specific viral antigen and to proliferate in its presence. The LBT response occurred more frequently in individuals with recent (<1 year) influenza B virus infections than in those with evidence of only remote (≥3 years) infections. Similar response patterns have been observed after infection with influenza A viruses (H1N1 and H3N2) (6–8, 14, 16), but there are no comparable data on responses after influenza B virus infection in humans. Influenza A virus-induced LBT responses were reported after immunization of adults and children with inactivated influenza A vaccines (6, 14). These vaccine-induced LBT responses were short-lived in both children and adults. We also observed short-lived LBT responses when evaluating young children recently infected with an influenza A (H1N1 and H3N2) virus (unpublished data).

Lazar and Wright reported the persistence of a low-level A/USSR (H1N1) LBT response in adults who, they suggest, had not been infected for more than 20 years (15). This may have resulted from repeated exposure to other type A influenza viruses (4). In our study population, the failure of the influenza B LBT response to persist in the majority of remotely infected adults and children could represent a difference in the LBT response to type A and B viruses, or it may be related to an absence of cross-reactive stimulation between subtypes as reported by others (4).

Little protection against infection with influenza B virus was demonstrated during a 3-year follow-up in children given an inactivated vaccine, despite the persistence of influenza B Ab (9). This lack of efficacy may have been related either to the lack of persistence of a cellular immune response or to the failure of such a response to develop.

There was no correlation between age at the time of reinfection and the LBT responses of our subjects, although there was a trend toward more frequent LBT responses in the reinfected adults than in the reinfected children. No correlation was noted between the Ab titer and influenza B virus-induced LBT, in agreement with findings of others (3, 7, 16, 17). The appearance of LBT responses preceding an Ab response in four children has also been reported after influenza A virus vaccination (8, 15) and may be useful in demonstrating an immune response to influenza virus infection in the absence of a serological response.

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LITERATURE CITED


