Studies of the Blastogenic Response of Murine Lymphocyte III. Specific Viral Transformation (36726)

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A variety of leukemias and lymphatic diseases have been associated with a decreased immunological response of the host. This has been reported in mice infected with oncogenic viruses (1, 2) and in humans diagnosed as having certain malignancies (3, 4). Depressed delayed hypersensitivity has been observed during measle virus infection of man (5). Similarly decreased reactivity to tuberculin sensitization of man has been noted with myxo, pox and picornavirus infections (6). The addition of measles, rubella, newcastle disease, polio, influenza and other viruses, directly to human lymphocyte cultures, has been reported to depress lymphocyte transformation (7). Olson, South and Good (8) have noted that lymphocyte transformation was depressed in infants infected with rubella virus. Such an effect has similarly been noted in patients with infectious mononucleosis and infectious hepatitis (9), both diseases of probable viral etiology. Whether viral infection depresses host lymphocyte transformation response in general or selectively affects specific lymphocyte populations has not been adequately evaluated. Viral induced changes in macrophage and lymphocyte surface structure and/or metabolism may alter the ability of these cells to respond to both specific and nonspecific transformation stimulators. We present evidence which suggests that either specific lymphocytes or certain sites on murine lymphocytes are committed to single viral stimuli, this observation had previously been made by Zoschke and Bach (10) in another lymphocyte-stimulator system. We present evidence further demonstrating that even though a mouse may be immunologically depressed by leukemia, it can maintain a limited capability to respond to specific and nonspecific lymphocyte transformation.

Materials and Methods. Weanling female Balb/cAnN mice were divided into four treatment groups: intraperitoneal (ip) infection with 10^{-3} Rauscher leukemia virus (RLV), intranasal (in) infection with a LD_{30} dose of PR-8 influenza virus, coinfection with PR-8 (in) followed in seven days by RLV (ip) and a group of control mice. To demonstrate mouse strain variability in lymphocyte response to specific viral stimulation, C_3 H/ENT mice were also investigated, but only with PR-8 sensitization.

Culturing procedures for mouse lymphocytes have been described previously (11). Cultures containing 10^6 mouse splenic cells/culture were incubated with either 1 μl of PHA-P (Difco), a 10^{-3} dilution of 10% suspension of PR-8-infected mouse lung tissue or a 10^{-3} dilution of Rauscher leukemia virus prepared in sodium citrate. In all instances the virus stimulant was prepared in the same strain of mouse and appropriate normal spleen and lung tissue were used for control experiments. Those animals infected with PR-8 virus were used as lymphocyte donors only if a positive lung consolidation was evident. In all cases at least three spleens from each treatment group were pooled for lymphocyte culture preparation.

Cultures were stimulated at the onset of incubation and isotopically pulsed 24 hr after the addition of PHA or 48 hr after the addition of virus. Twenty-four hours after the addition of 0.1 ml of medium containing 2 μCi/0.1 ml of ^{3}H-thymidine (sp act, 1.9 Ci/mmole) the cultures were frozen until subsequently processed to determine the incorporation of radioactive thymidine into the

acid-insoluble pool (11).

Results and Discussion. A low and high responder mouse strain was selected from 6 strains on the basis of their PHA induced lymphocyte transformation response which is depicted in Fig. 1.

It is noted in Fig. 2 that the C_3H mouse lymphocytes *in vitro* respond to PHA stimulation to a higher degree than to PR-8 stimulation, however the response of mice previously challenged by PR-8 virus infection in general is greater to this stimulus than the response observed in mice not previously exposed to PR-8 viral infection. The more vigorous PR-8 response of the Balb/c mouse is consistent with their greater response to PHA stimulation (Fig. 3) as might be expected. The pattern of response to PR-8 stimulation in general corresponds well with the pathological phase of the animals response to PR-8 infection. In general, animals begin to demonstrate a decrease in alveolar hyperplasia corresponding to the time that we note a depression in their lymphocyte stimulatory response. This *in vitro* lymphocyte responsiveness could not, however, be correlated with any consistent pattern of interferon response. The significance of this high stimulation index observed at 35 days may indicate a secondary tissue sensitivity response to hyperplastic lung tissue.

As shown in Fig. 4, lymphocytes from Balb/c mice infected with RLV, although depressed when compared to the PHA response observed in the absences of RLV infection (Fig. 1), nevertheless showed the capacity to be significantly stimulated by PHA but not by RLV.

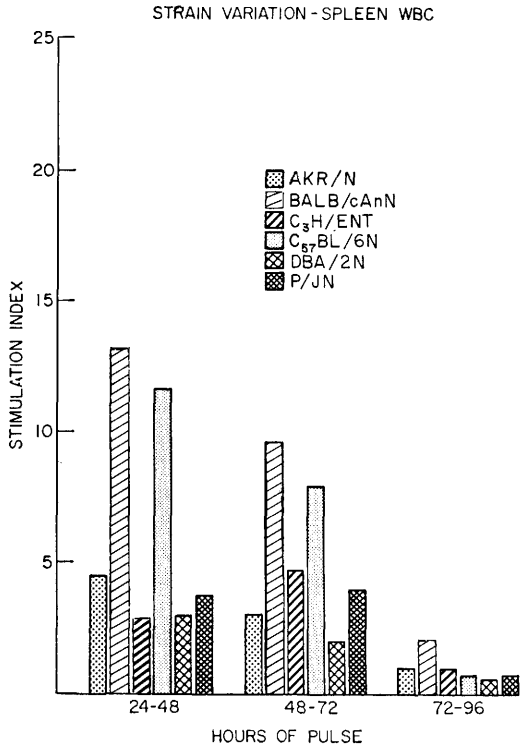

FIG. 1. Influence of mouse strain variability on PHA-stimulated lymphocyte blastogenic response.

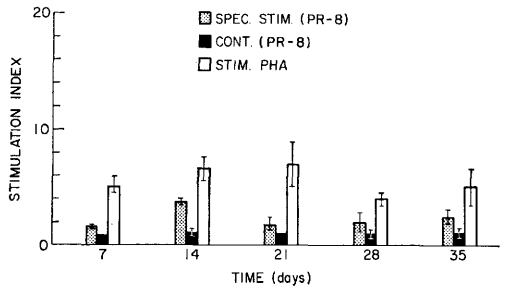

FIG. 2. C_3H/ENT mouse splenic cell blastogenic response to PR-8 influenza virus and PHA stimulation.

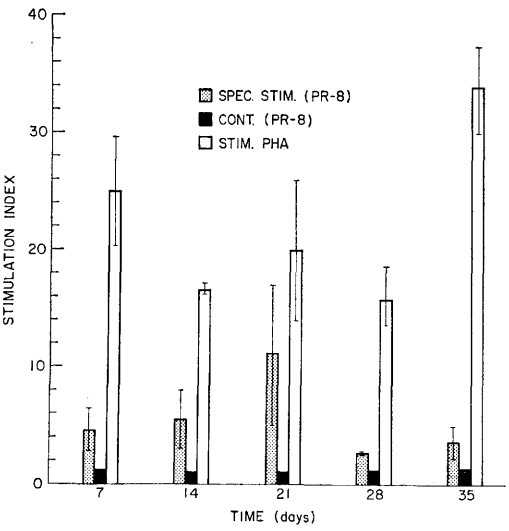

FIG. 3. BALB/c mouse splenic cell blastogenic response to PR-8 influenza virus and PHA stimulation.

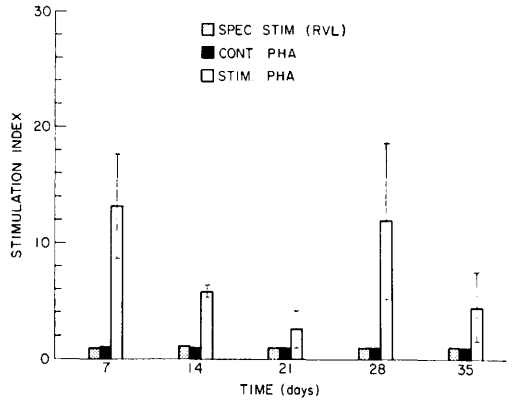

FIG. 4. BALB/ mouse splenic cell blastogenic response to Rauscher leukemia virus and PHA stimulation.

Of particular interest is the response of Balb/c mice coinfected with PR-8 and RLV. As shown in Fig. 5, lymphocytes obtained from splenic tissue of mice coinfected with both viruses showed a reduced but significant PHA and PR-8 stimulation when compared to Fig. 3. Interestingly RLV did not elicit any lymphocyte stimulation regardless of whether PR-8 infection preceded or followed RLV infection. These observations demonstrate that utilizing blast transformation as an indicator of cellular immunity, as has been suggested by Oppenheim (12), animals that appear immunologically depressed for one stimulus are still capable of responding to other specific or nonspecific stimulants. Hanna *et al.* (13) have demonstrated that nonthymic dependent areas of the lymphatic tissue are the primary sites of RLV replication and Hanna, Szakel and Tyndall (14) showed that at a later stage of RLV infection, the cortex but not the medullar region of the thymus shows budding of C-type RNA virus particles. These observations may explain the apparent deficiency of lymphocytes from RLV-infected mice to recognize this virus as an *in vitro* antigen since the limited number of cells responsible for transformation (15) may never have been exposed *in vivo* to the antigenic sensitization. As an alternative explanation, it is possible that the host does not immunologically recognize C-type RNA tumor viruses as foreign antigen, since the latter incorporates host cell membrane during the process of virus release.

A series of experiments designed to compare transformation capabilities of splenic and peripheral lymphocytes from animals infected with PR-8 and RLV showed that peripheral lymphocytes do not respond to either RLV or PR-8 stimulation. In contast to observations made by Hayry, Rago and Defendi (16), we noted spleen-derived lymphocytes of RLV-infected mice showed reduced but significant PHA-induced transformation.

Despite the immunosuppressive effect of RLV on a variety of immunological parameters (17), our data suggests that the spleen cells of RLV-infected mice consist of several cell populations, some capable of responding to specific PR-8 virus stimulation, while a larger proportion of cells respond to PHA stimulation. Data published by Borella (18) lends itself also to the interpretation that several cell populations may be involved in the cellular and humoral immunological response. We have demonstrated that RLV infection does bring about an appreciable degree of immunosuppression as indicated by a reduced but still significant PHA response. The difference in magnitude of the general PHA response versus the specific PR-8 response would further suggest that the host has a sufficient number of uncommitted cells available that are capable of recruitment for subsequent specific antigen commitment.

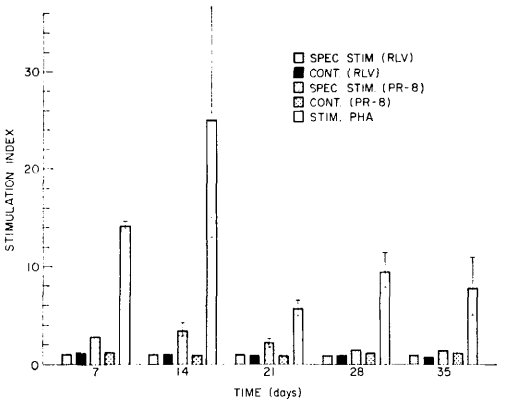

FIG. 5. BALB/c mouse splenic cell blastogenic response of mice coinfected with Rauscher leukemia virus and PR-8 influenza virus, blastogenic response to PR-8, RLV and PHA.

Similarly the reduced response of the host cells to PR-8 sensitization also demonstrates a RLV suppressive effect. However, in both the PHA and PR-8 stimulation parameters, the host still has a sufficient number of responsive cells to elicit demonstrable blastogenesis.

Summary. Data presented suggests that either specific lymphocytes or specific sites on such cells are committed to both specific and nonspecific lymphocyte transformation responses. Though the animal may be immunologically depressed by leukemia virus infection (RLV), its lymphocytes are still capable of a limited but significant cellular immune response to specific viral and PHA stimulation.

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