Formal Discussion: Immunologic Aspects

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Dr. Kaplan has succinctly and lucidly delineated the areas in leukemia research that require greater understanding if scientifically derived measures for the control of leukemia are to be obtained. Immunologically speaking, the 2 major divisions of control—prevention and cure—are extremely useful in one's approach to the problem, for they separate 2 distinctly different immunologic mechanisms, which may, perhaps, be exploited in immunotherapy.

IMMUNOTHERAPY OF ESTABLISHED LEUKEMIA

As a guide to our understanding of the serotherapeutic approach, let us consider a few of the many events that might follow the application of immune sera. Although a more fully detailed and documented account of these events will appear later (3), a brief description seems to be in order at this time.

Antigenic deletion without cytocide.—There is precedence for this in the recent work of Boyse *et al.* (1). The C57BL/6 leukemia, ERLD, contains the specific leukemic-cell antigen, TL, and specific anti-TL-antibody is cytotoxic for ERLD cells *in vitro*. When the leukemia is transplanted into C57BL/6 hosts, pre-immunized with TL-antigen, growth is *not* inhibited. Instead, TL-antigen is deleted from the tumor. Anti-TL-antibody is now no longer cytotoxic *in vitro* for the tumor, nor is the tumor effective in absorbing anti-TL activity *in vivo* or *in vitro*. In spite of this, when the tumor is transplanted to a non-immune environment, TL-antigen reappears in full on 1st passage.

Cytocide of endogenous cell regulator.—Leighton (7) has derived a theory of interclonal equilibrium as a physiologic regulator of tumor growth from his searching studies on the interaction of cell populations. A tumor is visualized as a family of clones, in which the slowest growing is the endogenous regulator. When the slowest growing clone is lost, increased anaplasia, increased rate of growth, and increased capacity to metastasize on the part of the remaining clones become manifest. Selective attack upon the slowest clonal member, resulting in its loss, would therefore be hazardous to the host.

We know, from the work of Hirszfeld *et al.* (6), that distinctive cancer antigens are plentiful in tumors that are only slightly anaplastic, whereas they are almost completely absent in the extensively anaplastic ones. In such cases specific immune attack could easily be visualized as driving the tumor toward increased anaplasia. Although we do not know that leukemia would react in this manner, it is a point well worth keeping in mind. From this point of view, the most specific antiserum that could be directed toward a patient's leukemic cells would be the one against the least malignant clone. Its therapeutic application would result in loss of the least anaplastic clone and enhancement of the remaining clones.

Normal-tissue injury.—Following the formation of nonspecific, serum-borne, antigen-antibody complexes in experimental animals, Dixon (4) has observed that serum sickness, kidney damage, and other tissue injury result from the nonspecific accumulation of these complexes in certain juxtavascular areas. Host antibody is formed against the complexes, and at certain critical periods during the immune response it leads to the pathologic condition of normal-tissue injury. Day (2, 3) has shown that radioantibody, specifically localized in rat hepatoma, is rather unstable and that after it leaves the hepatoma it relocalizes in the kidney. The presumption is that specific antigen-antibody complexes are released and are subsequently relocalized in the kidney. Given this explanation for the observed data, one might well imagine that, during massive serotherapy of an existing malignancy, a side reaction of the therapy would be serum sickness, kidney damage, and other normal-tissue injury.

Leukemicide.—This is the desired event. To bring it about, specific immunologic attack upon the most anaplastic or most antigen-depleted clones would seem to offer the best likelihood for success. However, such an immunologic target would be the most difficult of all because of its presumed lack of distinctive antigens. To design such an antibody would require considerable stretching of one's imagination.

As one will recall, Seligmann *et al.* (11), in a beautifully controlled piece of work, found that chronic lymphoid leukemic cells contained only 2 of the 4 saline-soluble antigens that were contained in normal human leukocytes. Chronic myeloid leukemic cells lacked none of these. Distinctive antigens were not found in either leukemic state. Milgrom *et al.* (8) later found that the same antigenic distribution held for insoluble portions of the leukemic cells. Since the heteroimmune sera that were employed failed to detect isoantigens in the normal leukocytes, one wonders if, perhaps, distinctive antigens as well as isoantigens might more easily be found through the use of human isoantisera and if, in fact, the most effective antibody against a patient's leukemic cells might be his own against a cross-reactive substance from another human subject. The substance, because of its origin, would be isoantigenic but, because of its cross-reactivity, would virtually give rise to an autoantibody against the patient's own cells. Old, Boyse, and their associates are now exploring such a system with respect to murine leukemia.

There is little question that active immunization when possible would be preferred over passive immunization

in almost any circumstance. Adoptive immunotherapy, a variant to active immunization, as described by Dr. Mathé and his colleagues at this symposium, would also appear to hold greater promise.

Preventive Immunity to Leukemia

In the case of preventive immunity one would be trying not to treat existing leukemia with antibodies but rather to prevent its induction. Friend (5), for example, in a beautiful piece of work, treated filtrates of homologous Friend virus leukemic spleens with formalin to produce a vaccine. In adult Swiss mice the vaccine was effective in reducing the incidence of virus-induced leukemia from the original 80% to 20%. Old *et al.* (10) recently found that the incidence of Friend virus leukemia in adult BALB/c mice, induced by small amounts of virus, could be reduced 60% by the injection into these otherwise susceptible mice of excessive amounts of filtered live virus from Friend virus leukemic spleens of Swiss mice. Moreover, they found that the antisera from the protected mice were cytotoxic *in vitro* for Friend virus leukemic cells of unprotected BALB/c mice. The specific antigen involved was called FR because of its identity to leukemic antigens found not only in Friend virus-induced tumors but also in Rauscher virus-induced tumors. The antigen has since been found to share some cross-reactivity with a leukemic antigen produced by Moloney virus (9).

This last experiment provides us with a working principle that bears intensive investigation to determine whether it is limited or general. The priciple is this: Preventive immunity to a leukemia that is inducible by a given virus can in essence be produced by immunizing with an isoantigenic leukemia that is induced by the same virus. Since antisera against leukemic viruses are notoriously ineffective in controlling the incidence of leukemia, it is probable that the effective immunizing antigen is a cell product that is cross-reactive among individual leukemias induced by the same virus. The individual cell product, by virtue of its isoantigenic qualities, produces an antibody in another individual, which cross-reacts with that other individual's virus-induced cell product, i.e., to an autoantibody that effectively reacts with the host's own virus-infected tissues.

It is also possible that such an antibody may effectively neutralize the virus at its focal point of tissue alteration. If so, then the infected cells may revert to normal, nonneoplastic behavior, as envisioned by Dr. Kaplan.

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