Immunological Aspects

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Three immunological areas were represented in this session. The one dealt with the detection and characterization of leukemia-specific or leukemia-associated antigens; the second with the detection of antibodies directed against antigens associated with leukomogenic viruses in animals; the third topic dealt with two aspects of tumor-host relations.

1. Membrane Antigens of Leukemic Cells

Rodt and his associates described an ALL-associated antigen (or group of antigens) detectable by a xenogeneic antiserum reagent. This reagent, after absorption, reacted only with ALL cells but not with a variety of normal adult or fetal cells. Cells from 46 patients out of 66 assayed were shown to be positive. However, it would be desirable to obtain data on the biochemical or immunochemical nature of the antigens.

Greaves described this morning the occurrence of an ALL-associated antigen which, in view of its presence on certain fetal cells and in regenerating haemopoietic tissue, was described as a differentiation rather than an ALL-specific antigen. In spite of this prima-facie difference between the antigens described by the two groups, it would be of interest to find out whether or not the Rodt antigen corresponds to the differentiation antigen found by Greaves. It would be important to determine whether or not an absorption of Greaves' antiserum with cells originating from regenerating haemopoietic tissue would removenactivity against these cells but leave a residual activity towards the leukemic cells. This situation would fit that described by Rodt. On the other hand, the possibility cannot be excluded that by using more sensitive assays with their antiserum, the Rodt group will be able to detect activity with some fetal cells.

Kabisch and his colleagues discovered that an ALL-associated antigen circulates in leukemic patients. It seems that this glycoprotein antigen (molecular weight of 135 000) is shed from leukemic cells and reaches the circulation. As the characteristics of this antigen are very similar to those of the differentiation antigen on leukemic cells described by Greaves, it may be useful if these groups would exchange reagents in order to ascertain this point.

It may be of interest to determine if the ALL-associated antigen is immunogenic in humans. In view of its presence in the circulation it may not be easy

to detect the corresponding circulating antibodies. To answer this question it will probably be necessary to dissociate putative circulating immune complexes.

2. Antibodies Reactive With C-Type Viruses

Two attempts to find circulating antibodies reactive with C-type viral antigens in the normal human population were presented. Snyder's results, in conformity with those presented this morning by Kurth, indicated the presence of antibodies directed against exogeneous animal C-type viral antigens. Hogg and her colleagues, on the other hand, in a carefully executed study, were unable to detect such antibodies in human beings. Neither did they detect viral components on fresh peripheral leukocytes from normal individuals or from leukemia patients. The reason for this discrepancy is not clear.

Several points of technical or methodological nature are probably relevant in connection with these and similar findings.

Cross-reactivity and specificity: Investigators detecting serological reactivity against viral antigens must rule out the possibility that this reactivity stems from the exposure to heterophile cross-reacting immunogens present in human-associated bacteria, in ingested food, drugs, etc. This was indeed done by the present authors.

On the other hand, it should be remembered that a wide spectrum of cross-reactivity does not mean that the reaction is non-specific. In this connection it may be useful to consider any immune reactivity involving the active-site of the antibody molecule as a specific reaction. Thus, a more extensive use of $F(ab')_2$ fragments in serological assays would be encouraged.

Absorption: The studies summarized here and many of those reported elsewhere involve absorptions as an essential step in defining the specificity and cross reactivity patterns of antiserum reagents. Absorptions are valuable only if a full depletion of reactivity against the absorbing antigen was achieved. Any study involving absorptions should therefore include an assay to ascertain a complete and exhaustive absorption.

The Moroni group has previously shown that expression of endogenous C-type viruses in lymphocytes is increased as a result of a response to B cell mitogens. In another study they asked the question whether or not viral gene expression is physiologically required for an immune response. They could show that an antiserum directed against an endogenous xenotropic BALB/c virus suppressed the immune response of mice against sheep erythrocytes in-vivo as well as in-vitro. A new finding given in the poster presented by DeLamarter is that an antiserum directed against Friend leukemia virus acted as a B cell mitogen. The antibody performing this function was, however, not sufficiently characterized and its specificity not defined.

This work, as well as the studies of others showing that C-type viral expression on lymphocytes increases after an immune stimulation, may pos-

sibly explain antibody formation against such viruses in normal individuals. It is thus not unlikely that virus-associated antigens may reach immunogenic doses in immunized or mitogen-stimulated animals. Hence, an immune response against these antigens may actually be a result of a proliferative response of lymphocytes, either to unrelated antigens or to mitogens.

3. Tumor-Host Relations

The next two presentations deal with cellular immune functions of leukemic patients.

In the work of Knight and his colleagues, it was indicated that a certain proportion of patients with untreated acute phase myelogenous leukemia are non reactive towards allogeneic cells. Fractionation of leukocytes from non reactive patients on nylon wool columns yielded, in some cases, alloresponsiveness either in the adherent or in the non-adherent fractions. No explanation was offered for this phenomenon. Reconstitution experiments could show whether a suppressor cell population was separated or inactivated during the fractionation procedure.

Culturing of peripheral blood lymphocytes from leukemia patients in the presence of a factor present in conditioned medium brings about increased T cell functions of the cultured cells. This finding is of particular interest since human T-cell cultures are usually not easy to maintain.

The increased reactivity of the cultured T-cells from patients is in line with results of others showing that functions of lymphocytes from tumor-bearing individuals may be increased following culturing. This phenomenon may be due to the removal of blocking molecules from the membrane of the lymphocyte.

The purpose of the study of Oliver was to augment specific cellular reactivity of acute myeloid leukemic patients toward their malignant cells. To achieve this aim Oliver employed the principle of "Pool-priming" described this morning by Bach, namely, that generation of cytotoxic T-cells requires two types of cellular antigens, the LD and the CD determinants, which could even be present on different cells. It was found that Daudi cells, a lymphoblastoid cell line provided HLA D (LD) determinants which were apparently missing from the autologous blasts. Thus, the addition of Daudi cells to mixed cultures composed of remission lymphocytes and of autologous blasts augmented considerably the cytotoxic activity of the lymphocytes which were identified as T cells. Cold-target inhibition assays suggested that the target antigen was not present on the allogeneic Daudi cells.

Oliver also demonstrated that addition of lymphocyte interferon to the 3-cell mixed culture augmented cytotoxicity. The mechanism for this phenomenon is not known. It is of interest to mention that interferon seems also to augment the cytotoxic activity mediated by natural killer cells.

The work of Keisari, although not involving human leukemia, is of relevance to this Meeting as will be pointed out.

Some malignancies, including leukemias, evoke an immune response

against antigens associated with the malignant cell. It can be shown that as a result of this response certain immune effector mechanisms such as immunocytes or antibodies make contact with tumor cells in-vivo. For example, several authors have found that certain leukemia cells are coated in-vivo with Ig, possibly antibody.

In-vivo coated tumor cells seem to lose their surface-bound Ig upon transfer to culture conditions. The disappearance of the coating Ig molecules might be a result of three nonmutually exclusive mechanisms:

1. Endocytosis; 2. shedding; 3. degradation of the cell-bound Ig molecules by cellular proteases.

Keisari's findings indicated that tumors contain proteolytic enzymes capable of degrading anti-tumor antibodies as well as other IgG molecules in cell-free systems, and that the degradation products of the anti-tumor antibodies blocked lymphocyte-mediated and complement-dependent cytotoxicity at the target cell level. These experiments were carried out in cell-free systems and at a low pH. Further experiments showed that antibody-coated viable lymphoma cells under physiological conditions in culture were capable of degrading their antibody coat into low molecular weight degradation products. Lack of degradation of unrelated antibodies present in the culture medium suggested that under the experimental conditions employed, binding of antibodies to their target cells is an essential prerequisite for their degradation, and that degradation took place in the close vicinity of, or inside, the cells.

It is not unlikely that antibodies localized on the tumor cell, may be affected considerably by proteases originating in tumor cells. The results of Cotropia et al., showing that Ig molecules coating human leukemic cells are partially degraded, indicate that degradation of Ig by malignant cells can occur in-vivo. The most obvious consequence of such a degradation would be a continuous consumption of anti-tumor antibodies, resulting in their selective depletion.

The last paper to be reviewed is that of Joshua dealing with the definition of certain cell surface antigens of human leukocytes. He used Milstein's approach of producing hybridomas by fusing non-secreting murine plasmacytoma cells with cells producing antibodies to such antigens. This approach is being very rapidly introduced to all areas of immunology including, of course, tumor immunology.

In the present study the authors produced several cell-hybrids recognizing individual membrane antigens. They were also able to assign the expression of several surface antigens of leukocytes to particular chromosomes.