The Need to Establish Whether the Leukocyte Adherence Inhibition Test Is a Reliable Assay of Tumor Immunity in Humans

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Abstract

Lymphocytes from human cancer patients can react to tissue-type-specific antigens shared by many tumors of the same histological type and different for tumors of different types. Such reactions have been detected, for example, by using microcytotoxicity assays and leukocyte migration inhibition techniques. Work presented at this workshop indicates that the leukocyte adherence inhibition test is an excellent assay for detecting reactivity to antigens shared by neoplasms of the same histological type. However, very little is known of the nature of these antigens; for example, we do not know whether they are tumor specific or just normal tissue antigens. The usefulness of the leukocyte adherence inhibition test for patient monitoring and for diagnostic purposes also needs to be studied more.

Work published in the 1960's indicated that neoplasms of the same histological type share tumor-associated antigens, against which cell-mediated immune reactions can be detected. For example, it was reported that lymphocytes from tumor patients were specifically reactive in colony inhibition and microcytotoxicity assays to cells from short-term explants of allogeneic tumors of the type borne by the respective patients (6, 7, 9, 10). Since reactivity was found also in autochthonous combinations and when lymphocytes from patients with different types of cancer were tested against target cells of the respective type in a "crisscross" pattern, it was concluded that it was directed against some tumor-associated antigens rather than against normal histocompatibility antigens, and that it was not the outcome of "nonspecific" toxicity. Sera from patients with normal histocompatibility antigens, against which cell-mediated reactivity to the patient's own cultivated tumor cells and to allogeneic tumor cells of the same histological type (12), while sera from patients free of disease block only infrequently and often have a reverse effect (10). It was shown, furthermore, that reactivity detected by using the microcytotoxicity assay shows a rough correlation with the patients' tumor status in vivo if the blocking serum is taken into account and that testing of this reactivity has some prognostic value in that, among patients who were tumor free when tested, those who have blocking sera more often have relapses than those patients who do not (5).

Subsequent studies from many laboratories (1, 3, 15, 16, 18, 19) using primarily leukocyte migration inhibition techniques have confirmed the concept of cell-mediated immunity responses to antigens shared by tumors of the same histological type, although they have not revealed much about the nature of the antigens involved. This confirmation was much needed in view of the failure of several investigators to reproduce the original findings obtained with colony inhibition and microcytotoxicity techniques (14). The reason for this failure is unknown but may be, at least to some extent, that long-term cell lines which have been much used in the more recent work are more sensitive to the cytotoxic effect of natural killer cells than are short-term explants (4).

Although cell-mediated reactivity to tissue-type-specific human tumor antigens can be detected with the microcytotoxicity test, when "nonspecific" effects of natural killer cells are properly taken into account (2), neither this test nor the leukocyte migration inhibition technique is useful for monitoring individual patients. Sometimes lymphocytes react to antigens from tumors of the "wrong" histological type, and sometimes reactivity is not detected at all (13). There is, therefore, a great need for sensitive and easily standardized in vitro assays of cell-mediated reactivity to human tumors.

Many papers have been published lately claiming that the LAI test provides such an assay. They have been reviewed at this workshop, and new papers with a similar message have been presented there. (We are, therefore, not listing the many references to LAI studies in human tumors here.) The LAI technique has been reported to give very few false-positive and false-negative data and to be potentially useful for both diagnostic and patient-monitoring purposes. In view of the importance of these reports and also in view of the remaining skepticisms among many about cell-mediated immunity to tissue-type-specific human tumor antigens, it is necessary to settle how reproducible the findings obtained with LAI assays are and how useful the test is for clinical purposes.

Our own experience with the LAI test in animal and human tumors systems has been published. We will draw 2 conclusions from it. First, we have been able to confirm that LAI testing can be used to detect reactivity to tumor-associated antigens in animals (8) and in humans (11), including reactivity to antigens individually unique to different chemically induced sarcomas of mice and such that are shared by human tumors of the same histological type. Second, we have not observed the degree of reproducibility and specificity needed to indicate that the LAI test is a clinically useful assay of human tumor immunity; sometimes reactivity has been detected against the "wrong" tumor antigens, and sometimes we have failed to detect...
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reactivity against the "right" ones. Whether this is due to our insistence in including crisscross controls in essentially all experiments, to some deficiencies of the antigen preparations used, or to the test system itself is unclear. Since, however, negative experiments are less likely to be published than those reporting success, we will not be surprised if our somewhat disappointing experience with the LAI test has counterparts in other laboratories as well.

For this reason, we have felt a great need for double-blind studies to be performed to settle whether specific LAI data can also be reproducibly obtained under conditions when investigator bias can be totally excluded. As a first step toward this, one of us suggested a demonstration on coded samples to be held at this workshop. This suggestion was accepted.

Conclusion

Although the number of coded samples studied at the workshop was small, we are encouraged by the data obtained, particularly in view of the double coding procedures involved in the samples studied by Maluish and Halliday (17). The probability that chance alone was responsible for these findings is small, and we accept the conclusion, therefore, that the LAI test can be used to detect tumor-type related reactivity in human cancer patients. A need remains, however, to perform more coded tests on much larger samples of lymphocytes from various sources, including a large number of specificity controls, until it has been convincingly established that the precision of the LAI technique is as high as claimed. Some collaborative studies using frozen lymphocyte or sera (as in tests for blocking factors or cytophilic antibodies) may best serve this purpose, since they can be carried out in the investigators' own laboratories.

Even if the major claims made on the basis of LAI tests are correct (as we expect them to be), several important questions remain. First, what is the nature of the antigens involved? Are they shared by tumor cells and normal adult cells of the respective tissues, by tumor cells and normal stem cells, or by tumor cells and normal embryonic cells? Are the antigens truly tumor-specific, being present only on neoplastic cells and absent from various types of normal cells? Are some of them associated with some transmissible agents? Do they act as targets for an immune response in vivo, leading to tumor cell death, or are they, for example, intracellular components which are released normally or leak out when tumor cells are damaged? The very first steps toward solving these problems have just been taken, as discussed at this workshop.

Another question concerns how useful the LAI test is for diagnosing patients with tumor and/or monitoring their disease. Although encouraging data were presented at the workshop, conclusions on this must await the double-blind, prospective studies. One should realize, of course, that even if any antigens acting as targets in the LAI assays would prove to be normal tissue components, they might be still useful for monitoring cancer patients or in helping to provide diagnosis.

References


Discussion

Dr. Powell: I should like to address the HLA question which you raised. We have already presented or are publishing data indicating that in females with a history of pregnancy, particularly multiple pregnancies, we do, in fact, occasionally find positive responses with breast cancer antigen and also occasionally with extracts of peripheral blood lymphocytes, which is our control in that situation. Therefore, I think there certainly is such a thing as HLA sensitization which you can pick up by the LAI system, but I do not think that this accounts for the apparently specific reactions of extracts.

Dr. K. E. Hellström: No, neither do I.
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