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Perspectives and Commentaries

Leukocyte Adherence Inhibition: History, Biological Basis and Clinical Usefulness

ROBERT L. NUTTER and JAMES D. KETTERING
Department of Microbiology, Loma Linda University, Loma Linda, CA 92350, *U.S.A.*

(A COMMENT ON: Moskaug J, Kotlar HKr, Sanner T. Cell populations involved in the humoral leukocyte adherence inhibition reaction. The T8-suppressor/cytotoxic cells are both effectors and responders. *Eur J Cancer Clin Oncol* 1987, **23**, 149–159.)

LYMPHOKINES, soluble products with many biological activities, are secreted when stimulated lymphocytes from sensitized donors are exposed *in vitro* to specific antigens [1]. These substances have the ability to act on other cells and affect their activity. Inhibition or, less frequently, enhancement of mononuclear cells and neutrophils are mediated by lymphokines, for example. It was in attempts by Halliday and Miller (1972) to shorten the usual 18- to 24- hr incubation times in macrophage migration inhibition assays (which lymphokine has been called Migration Inhibitory Factor—MIF) that the Leukocyte Adherence Inhibition (LAI) test began to be developed. LAI depends upon the ability of a lymphokine, produced as a result of the exposure of leukocytes to an antigen to which the host has become sensitized, to cause the leukocytes to lose their ability to adhere to glass or plastic surfaces.

In their original LAI work Halliday and Miller [2] used peritoneal cells (PC) from mice with methylcholanthrene-induced fibrosarcomas. The PC were mixed with the specific tumor extract or an unrelated extract and were added to a hemacytometer chamber. After a 1 hr incubation period the cells were counted, the coverslip was slid off, the slide was dipped in culture medium to remove non-adherent cells, and the adherent cells were counted in the same squares. The PC from tumor-immune mice had a lower adherence to the slide in the presence of the corresponding tumor extract than with unrelated extract. They next experimented with human tumors beginning with a breast carcinoma. Blood leukocytes from the patient were mixed with extracts of the tumor obtained at surgery on the same day. This original method, with few refinements, is used to the present time in the direct hemacytometer LAI technique for the quantification of cell-mediated immunity. It has been especially applied to measure recognition of tumor-associated antigens in cancer patients.

An 'International Workshop on Leukocyte Adherence Inhibition' was held at Roswell Park Memorial Institute, Buffalo, New York, May 15–17, 1978. There were 19 participants and 22 observers. The papers presented, together with the discussion which followed, were published in *Cancer Research*, Vol. 39 (2, part 2) February, 1979. By the time the workshop was held over 60 publications on LAI had appeared. Some of these presented variations in the technique in which surfaces other than that of the hemacytometer were used for adherence. In one such variation the mixture of peripheral blood mononuclear cells and tumor extract is incubated in horizontal glass test-tubes [3, 4]. This has been called the test-tube LAI assay to distinguish it from the hemacytometer LAI assay. In another variation by Holt and his colleagues [5] the wells of microtest plates are used for the incubation of the leukocyte–antigen mixtures. This has been referred to as the microplate assay.

It was also Holt who first gave the name Leukocyte Adherence Inhibition Factor (LAIF) to the lymphokine responsible for inhibiting the adherence of the cells to the solid surfaces. In contrast

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to the direct LAI techniques previously described, an indirect technique was developed as a 2-step assay. Mixtures of the leukocytes, antigens, and serum are incubated for 1 hr as before. After centrifugation at approx. 500 ***g*** for 5 min, the supernatants containing the presumed LAIF(s) are removed and may be stored frozen until the second step of the assay, if desired. Later, aliquots of dilutions of the supernatants are mixed with the sensitized leukocytes and inhibition of adherence assessed as in the direct method. The use of dilutions allows determination of the titer of LAIF(s). A description of many of the LAI techniques has been contributed by Borish *et al.* [6].

In an effort to avoid the necessity to count cells in the LAI test, Tsang *et al.* [7] developed a technique in which the peripheral blood mononuclear cells were labeled with ^{51}Cr before use in either direct or indirect LAI assays. Incubations of mixtures were performed in tissue culture tubes. After 2 gentle washes, the tubes were inverted to drain on filter paper and were counted in a gamma counter after being allowed to air dry.

The article by Moskaug, Kotlar and Sanner [8] is the latest in a series of articles from the authors' laboratory and utilizes yet another variation of the LAI assay procedure. This was first described by Kotlar and Sanner [9]. Although the assay is performed by the hemacytometer method of Halliday and Miller [2], the authors name it the Humoral Leukocyte Adherence Inhibition (H-LAI) assay in contrast to the conventional hemacytometer test which they have identified as the cellular LAI or C-LAI. In the C-LAI assay the patient's own activated leukocytes are incubated with the appropriate tumor extract and the lymphokines produced reduce the adherence of the leukocytes. In the H-LAI an aliquot of the cancer patient's serum is incubated at 4°C for 1 hr with the appropriate antigen or tumor extract, after which trypsinized leukocytes from healthy individuals are added and the mixture is incubated at 37°C for 30 more minutes. Aliquots of the cell suspension are then transferred to hemacytometers where the rest of the assay is similar to that for C-LAI. There is evidence that complexes formed between the serum factor and tumor-associated antigen trigger the release of the lymphokine(s) responsible for reducing the adherence of the cells, peripheral blood leukocytes from normal individuals.

THE BIOLOGICAL BASIS OF THE LEUKOCYTE ADHERENCE INHIBITION ASSAY

Other variations of the LAI technique appear to have shown greater involvement of cells of the monocyte series and may measure different aspects of the immune response. This is particularly true of the tube LAI assay (which some have designated as the T-LAI assay) in which inhibition of adherence appears to be mediated by cytophilic antibodies which bind to the Fc receptor of the macrophages or monocytes. In order to avoid additional complexities, the following discussion will be limited to the C-LAI and the H-LAI tests.

Early experiments with the C-LAI assay, to define some of the mechanisms involved, utilized cell separation and identification techniques. Using lymph node cells from rats immunized with DNP-bovine gamma globulin and peripheral blood leukocytes from human volunteers immunized with keyhole limpet hemocyanin (KLH), Powell and his co-workers [10] convincingly showed that E-rosette-forming T-lymphocytes were necessary in the C-LAI assay and that the T-cells produced the lymphokine LAIF.

In a subsequent article [11] they showed with the KLH system that the cells which both produce the lymphokine and which respond to it in the C-LAI are T-cells that have receptors for the Fc portion of IgG molecules and which lack receptors for the Fc portion of IgM molecules. Meanwhile, Kotlar and Sanner [12] had shown that the subpopulation of trypsinized normal T-cells producing the lymphokine in their H-LAI assay was the group with surface receptors with affinity for immune complexes, i.e. Fc receptors.

One of the principal contributions made in the article under discussion [8] is that evidence is presented that T_8-cells are the sub-population of T-cells which respond to the serum factor–tumor antigen complex in the H-LAI assay to produce a lymphokine. Coupled with this is evidence that the cells which respond to the lymphokine and lose adherence are also T_8-cells. These findings make it possible to compare the cells and the mechanisms involved in the H-LAI test with those in the C-LAI test. Perhaps, whereas the trypsinized normal T_8-cells in the H-LAI assay have Fc receptors for the IgG-like portion of the serum factor–tumor antigen complex, the T_g+ cells from sensitized individuals in the C-LAI assay have Fc receptors which, in a sense, already possess the Fc portion of the IgG-like molecule and are stimulated to produce the lymphokine (LAIF) when they come in contact with the tumor antigen. In each of the 2 types of assay it is likely that the number of cells that lose their adherence is much greater than the number that react primarily with the complex or with antigen and that the lymphokine release is required to amplify the response.

Another significant contribution in the article of Moskaug, Kotlar, and Sanner is the finding that

depletion of monocytes from the indicator cell population enhanced the H-LAI reaction. Suppressor effects have also been reported in other immunological reactions, some of which also show involvement of T-cells carrying a T-suppressor cell marker [13].

LAI—CLINICAL USEFULNESS

While the LAI assays have been found to be reliable indicators of cell-mediated immunity, the actual use of these apparently has been largely restricted to the detection of immunity to tumor-associated antigens and the presence of blocking factors. Even though one variation, the H-LAI, detects an early indicator of at least 1 form of malignancy [14] years before tumor development, early expectations for this technique as a diagnostic tool have not been realized. The assays have not been developed to the stage where convenient kits are available. These would make the test readily available to the diagnostic laboratory. Currently the assay is used in only a limited number of research laboratories.

There appear to be several identifiable problems that will have to be addressed before the LAI test will be available on a wide basis. First of all, several basic forms of the test assay have been described and reported in the literature as mentioned earlier. While the C-LAI is limited to testing fresh blood samples, because it is dependent on the use of sensitized peripheral blood leukocytes from the patient, the H-LAI can test samples that are fresh or have been frozen. Also, the H-LAI would appear to be of advantage since lymphocytes from any human donor can be used as an assay ingredient after a trypsinization treatment. The ability to substitute this form of indicator cell would help to standardize the test parameters and probably result in a more precise test. The 2 tests appear to have approximately the same sensitivity, but the H-LAI may be more specific. Such improvements might allow the H-LAI assay to monitor antitumor therapy with considerable reliability.

One disadvantage that confronts the ready acceptance of this general test procedure is the relatively high cost of performance. The procedures remain, at this time, research laboratory techniques. Performance of the assay requires large amounts of technician time to prepare for the test, incubate, count and analyze the results. If some form of automation could be utilized, the cost factor could be reduced. Lack of standardization of the test materials also decreases the cost effectiveness and 'run-to-run' reliability of the assay. When tests remain at this level of performance, there can also be the disadvantage of a long time period before results are available. Clinicians often want test results back within a reasonable time to use them in patient management. Until the test can be designed so as to be readily available for performance in the clinical laboratory of the treatment center, the turn-around time for such reporting will probably be prolonged.

Another problem lies in the preparation of the tumor antigens used in the assays. While the procedure for the extractions are fairly well described, a great deal of variation may be introduced into the test, causing possible complications in interpretation. Use of several antigen preparations from the same tumor would probably be advisable at present. This would increase cost, but would decrease the variation in results. One indication of such problems is the advisability of titrating each antigen preparation before inclusion into the assay procedure [15].

While there may be problems which limit the usefulness of the LAI assay, the promising results described in the literature may indicate that this test could be adapted to widespread use in the diagnosis of malignancies if the problems can be solved. Certainly there is currently a great need for a reliable and specific laboratory test that will perform this role for the clinician. The tests that are routinely available today for tumor diagnosis are usually non-specific in regard to tumor type. An LAI test could provide specificity. Equally needed are tests that can be used to monitor the effects of antitumor therapy. Especially useful would be tests that could measure, with accuracy and reliability, increases in immune competence in direct response to a therapy being employed.

The LAI assay might serve as a significant screening procedure for certain types of tumors (breast or lung, for example). While such tests need not be utilized on all patients, certain populations, identified as most at risk or as benefitting most from very early diagnosis, could be examined. If general antigens could be developed from such sources as cell cultures of tumor tissues, perhaps a range of malignancies could be tested for. The ability to detect such components as serum blocking factors would also be highly significant. Such detection would enable the physician to include treatments that might stimulate the immune system, in addition to the other therapies, in an effort to overcome the effects of these factors.

Although much remains to be done before routine clinical application is available, the LAI test, and especially the H-LAI test, shows considerable promise.

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