APPLICATION OF COMPUTER ANALYSIS TO IMMUNOLOGICAL MONITORING ASSAYS

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Immunological monitoring of transplant patients represents a new approach to clinical management of immunosuppressive therapy in which the drug dosage is individualized on the basis of the results of these in vitro assays. The assays utilize multiple controls and triplicate experimental measurements requiring a handling of a large data base. To offset this problem, our laboratory has developed a computerized data calculation and storage system which utilizes an HP-3000-II series computer, BASIC language programming and a direct-interfacing punch tape reader from beta and gamma counters to calculate the killing of donor cells by recipient cell subpopulations. This technique has been shown to reduce data error, save technician time, reduce the time from blood drawing to the availability of test results and to achieve a marked saving in the cost of laboratory operations.

Introduction

Immunological Monitoring (IM) utilizing in vitro assays of immune reactivity has attracted considerable attention and interest among immunologists and clinicians managing transplant and tumor patients as well as patients with infections and sepsis in which assessment of the status of the immune system may provide valuable guides to the treatment or prognosis for the patient. In the case of transplant patients, IM techniques are designed to tailor to the dosage and types of immunosuppressive drugs to the individual recipients rather than use the traditional stereotyped drug regimens. These stereotyped regimens are known to result in a high incidence of "undersuppression" of the recipients leading to acute graft rejection as well as frequent "oversuppression" of immune reactivity resulting in serious infectious complications (1). Recent advances in in vitro techniques of IM have permitted the application of a variety of assays in clinical practice. At present, the following immunodiagnostic assays are performed on our kidney and heart transplant recipients: 1) MLC-mixed leukocyte culture in which the incorporation of tritiated thymidine in recipient cells responding to donor cells is measured following a five day culture and counted in a gamma counter; 2) CML-cell-mediated lympholysis in which the level of in vitro generation or recipient cytotoxic T effector cells to donor is measured by radioactive counting techniques; 3) LMC-lymphocyte-mediated cytotoxicity in which the direct in vivo generated T cell cytotoxicity to donor cells is measured by counting 51Cr release; 4) ADCC-antibody-dependent-cell-mediated cytotoxicity in which the cytotoxicity of so-called K+ cells to donor cells is measured by release of 51Cr from labeled donor target cells; 5) suppressor cell studies in which the ability of both adherent and non-adherent recipient cells to suppress the in vitro generation of cytotoxic T cells from an HLA identical sibling in response to stimulation by donor cells is measured by radioactive counting; 6) Serum Blocking Factor (SBF) studies in which measurement of the degree of reduction of recipient reactivity to donor cells following the introduction into culture of recipient serum. These SBF are also referred to as enhancing antibodies which are thought to promote the survival of allografts and the spread of tumors in some cases. The techniques of performing these tests are described and referenced in another article (2) and will not be further described here; 7) measurements of circulating T and B lymphocyte levels and the reactivity of T and B lymphocytes as previously described (9); 8) measurement of "K" cell activity by studies of cytotoxicity generated by recipient cells with a known positive ADCC antibody system (6).

Serial measurement of these in vitro parameters are performed at periodic intervals varying from daily to monthly depending upon the status of the patient and the time post-transplant. All of the studies involve at least three measurements which need to be averaged. In addition, the cytotoxicity studies complete with controls generate from 4 to 96 parameters which need to be entered into control formulas and a formula for the % specific cytolysis (Bunnen Equation) which reads as follows:

$$\% \text{ cytolysis} = \frac{E_{\text{max}} - S}{E_{\text{max}} - S}$$

where

- $E$ = experimental radioactivity in c.p.m.
- $S$ = spontaneous (control) radioactivity release
- $E_{\text{max}}$ = maximal radioactivity release with 4 cycles freezing and thawing of cells

After a four year experience with these tests, it became obvious that the calculation of test results as well as storage of serial information and correlation of in vivo in vitro parameters might be helped by computerizing this data. In this pub-
lication, we describe the results of a one year experience with this system is utilized to study 168 transplant patients using one or more of the described assays.

**Materials and Methods**

Techniques of kidney and heart transplantation as well as some general remarks about current systems of IM in these patients is available in recent publications from our group (3) (4) and will not be further detailed here. The techniques of performing the described in vitro tests have also been outlined in some of our previous publications and the interested reader is referred to these articles for further details of performance of these assays (5) (6) (7) (8).

Computerization of the assay results was accomplished using a Hewlett-Packard 3000-II series computer which has an on-line memory capability of 94 Megabytes. Data input was either manually or by utilization of a punch tape interface device which automatically enters generated data from the radioactive counters into the computer terminal. Means and standard errors of all triplicate measurements were calculated by the computer and either directly read one from a paper recorder or entered into a BASIC program for calculation of cytotoxicity and cytotoxicity controls. The BASIC program was designed in our laboratory and is available to interested investigators upon request.

Off-line data storage was performed utilizing a program which provides information on the dates of testing and certain clinical or in vivo parameters to allow correlation of in vitro test results with in vivo parameters of graft function, infection and other significant clinical events. Data retrieval was accomplished by standard techniques.

**RESULTS**

Figure 1 compares the relative time from the drawing of patient blood until test results are available to the clinician for decision - making using systems of manual and computerized data calculation. As noted, some IM assays such as T cell levels (shown on the left of the figure) can be calculated and entered as rapidly by manual means as by computer techniques (p>0.10). Thus, no significant advantage of computerized data is evident with this test. In contrast, as noted on the right side of the figure, the time required for ADCC and LMC cytotoxicity assays was significantly less using the computerized data handling techniques. The mean time for manual data generation and calculation was 2.6±0.7 days for ADCC cytotoxicity and 3.1±0.5 days for LMC cytotoxicity. The respective times for computerized data generation and handling were 0.7±0.16 days (ADCC cytotoxicity and 0.8±0.25 days (LMC cytotoxicity) (p<0.05 for both assays). Thus, the computerized data calculations permitted the availability of data two days or more earlier than the manual system in most cases. When times were compared for the CML assays, even larger differences were apparent between the computer system (6.8±0.3 days) and the manual system (12.8±1.9 days). In the case of suppressor cell studies, the largest differences were seen. The computer system (mean of 7.25±0.3 days) compared favorably (p<0.01) with the mean time of manual data gathering and handling (16.3±5.6 days). In the case of MLC results, the computer time of 5.7±0.2 days was superior (p<0.05) to the manual time of 9.6±2.2 days. Thus, for the MLC, CML, LMC and ADCC assays, the computerized system demonstrated significant saving of time.

Figure 2 demonstrates the incidence of significant errors in calculation of data using manual and computerized techniques. In this graph, 64 separate determinations calculated manually were compared with simultaneous computer calculations. Repeat calculations identified the true test value and the source of error. As noted, the mean incidence of calculation errors in excess of 5% of the true value using the computer technique was 0.2% compared to 16% using manual techniques. The incidence of error with the manual techniques varied widely from a low of 6% to a high of 26% which seemed to be roughly proportional to the complexity of calculations. The incidence of significant error was lowest with the T and B cell level assays intermediate with T and B cell reactivity assays and highest for the cytotoxicity assays.

**Discussion**

These results indicate that computerized techniques for data calculation and data handling of the parameters of IM assays were superior to techniques of manual data handling in at least two significant respects. Firstly, the time required to obtain assay results was significantly decreased with the computerized system. This has the advantage of a large saving of technician time and labor expenses for manual calculations. In addition, the more rapid availability of results permits more timely therapeutic interventions by the clinical staff. This was an important feature in permitting the high rate of diagnosis of a pre-rejection state by three monitoring assays used in conjunction with each other as previously reported (9). The mean time of diagnosis of a pre-rejection state was 5.8 days prior to the onset of clinical rejection. This time will apparently permit adequate modulation of immunosuppressive therapy and reduce the incidence of early acute rejection (10).

The lowered incidence of errors in data analysis using computerized techniques has been commented upon by a number of workers in the computer field (11). This contention was borne out in the present studies in which the incidence of error in data calculation was reduced 80-fold by application of computer analysis. In particular, the more complex calculations such as ADCC, LMC, CML cytotoxicity and suppressor cell data showed extremely large reductions in calculation errors with the use of computerized techniques. This reduction in errors of calculations is important in the reproducibility and validity of immune monitoring assays. The cytotoxicity assays, in particular, benefit highly from application of computer techniques since the complexity of calculations and the need for multiple controls render the assays highly vulnerable to potential errors.
In addition, these assays are often positive at a low level of specific cytotoxicity in the range of 5-15% and therefore, large errors in calculation can easily lead to false negative or false positive assays.

In our opinion, future computer design for calculation and handling of IM data can benefit from these early experiences. In particular, we would advocate the following general directions: 1) increasing reliance upon computerized data calculations and data handling to facilitate and speed IM results for clinical use; 2) increasing reliance upon computerized storage of IM data for retrospective studies. Computerized data retrieval should be far superior to the conventional use of manual entry patient flow sheets which are cumbersome to store, have an inherent error factor present in every manual entry procedure and do not permit application of rapid mathematical techniques such as regression analysis, etc. without additional manual entry; 3) increasing use of techniques for direct interface of radioactive counters with computer input equipment to eliminate the need for manual computer entry which is time-consuming and a source of potential error; 4) increasing utilization of computer-generated data for making clinical decisions on immunosuppressive drug dosage according to in vitro IM assays. The use of individualized dosage of immunosuppressive drugs has been associated with a marked improvement in graft survival of transplant recipients in our clinic (10). This therapeutic modality is highly dependent on prompt and accurate results of IM assays which is aided by computer analysis.

**Summary**

Experience with immunological monitoring assays analyzed by both manual and computerized techniques has indicated a marked advantage in cost, time availability of results, accuracy of results and analysis of results using computerized techniques with a rather simple BASIC computer language system. This experience indicates that IM assays are highly relevant and useful clinically and that this utility is expanded by use of computer techniques for data analysis and storage.

**References**