Laboratory Support for Transplantation

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The clinical laboratory plays a major role in support of transplantation. Depending on the spectrum and number of transplants performed, such programs can consume up to 35% of all laboratory testing. This testing can be transplantation-nonspecific or -specific. The former constitutes routine laboratory procedures in various areas of laboratory medicine that are common to other categories of patients. Transplantation-specific testing includes tissue typing, procedures for assessment of graft viability/rejection, and measurement of immunosuppressive drugs. Because of the development of new immunosuppressive drugs such as FK506, cyclosporin G, and rapamycin, an increased burden will be placed on laboratories to establish new therapeutic monitoring programs. Preliminary guidelines for monitoring these drugs, similar to those previously established for cyclosporin A, are proposed herein. With the use of an increasing spectrum of immunosuppressive drug combinations, and the possible use of xenotransplantation on the horizon, new demands will be placed on the laboratory for the support of transplant programs.

Indexing Terms: immunosuppressive drugs/rejection markers/monitoring therapy/immunosassays

The number of transplant programs and transplants performed have increased steadily over the past 10 years (1). The main factor limiting further increases in transplant numbers is the chronic shortage of organ donors (1). Transplantation is now an acceptable medical procedure for treatment for end-stage renal, cardiac, lung, and bowel disease (2). The number of cellular transplants involving bone marrow and pancreatic islets for treatment of hematological abnormalities and diabetes, respectively, has also increased over this period (2). The evolution of both solid organ and cellular transplantation was made possible by the discovery and understanding of the human leukocyte antigen (HLA) system and the subsequent development of immunosuppressive drugs such as prednisone, azathioprine, cyclosporin A (CsA), OKT3, and tacrolimus (FK506).1 With the development of new immunosuppressive drugs such as mycophenolate mofetil (RS-61443), rapamycin (RAPA), and cyclosporin G (CsG) (OG37-325), it is hoped that improved graft survival with reduced drug-related side effects will be achieved.

The clinical laboratory has an integral function in the support of transplant patients that includes performing nontransplantation-specific tests (common to other patients as well) and transplantation-specific tests. Here I review the role of the clinical laboratory in these areas.

Nontransplantation-Specific Testing

Clinical laboratories are required to provide a broad range of services for transplant patients. These services encompass the various disciplines of laboratory medicine, including clinical chemistry, hematology, microbiology, blood bank, histology, and tissue typing. The required availabilities of these services for a transplant program are listed in Table 1. In most centers the renal transplant program constitutes the largest proportion of transplant patients. The pre- and posttransplant laboratory orders for a renal transplant recipient at the University of Alberta Hospitals are shown in Table 2. The distribution of testing among the various sections of laboratory medicine for all transplant programs at the University of Alberta Hospitals is shown in Fig. 1. The greatest impact of utilization is in the clinical chemistry and hematology laboratory sections.

The financial impact of transplant programs on the laboratory is significant. In a recent report the impact of a bone marrow and liver transplant program at the University of Nebraska was reviewed (3). Since the implementation of transplant programs at this institution in the mid 1980s, the University of Nebraska Medical Center experienced a 217% increase in procedure volume and a 165% increase in full-time equivalents as a result of these programs (3). Transplant-related procedures constitute ~35% of the testing volume at this institution. The laboratory procedures performed on patients undergoing transplantation generate ~$9 million per year.

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Table 1. Availability of laboratory services for a multiorgan transplant program at the University of Alberta Hospitals.

<table>
<thead>
<tr>
<th>Laboratory service</th>
<th>Days/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical chemistry</td>
<td>7*</td>
</tr>
<tr>
<td>Hematology</td>
<td>7*</td>
</tr>
<tr>
<td>Tissue typing</td>
<td>7*</td>
</tr>
<tr>
<td>Blood bank</td>
<td>7*</td>
</tr>
<tr>
<td>Microbiology</td>
<td>7</td>
</tr>
<tr>
<td>Therapeutic drug monitoring</td>
<td>7</td>
</tr>
<tr>
<td>Histology</td>
<td>5</td>
</tr>
</tbody>
</table>

* 24 h/day.

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1 Nonstandard abbreviations: CsA, cyclosporin A; CsG, cyclosporin G; FDA, Food and Drug Administration; FPIA, fluorescence polarization immunoassay; HLA, human leukocyte antigen; IL-2, interleukin 2; MEGX, monoethylglycinexilidide; MPA, mycophenolic acid; and RAPA, rapamycin.

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At the University of Alberta Hospitals, a 800-bed tertiary-care teaching hospital, ~80 renal, 25 heart, 30 liver, and 11 heart–lung transplants were performed in 1993. The relative laboratory costs per patient for the transplant programs on an arbitrary scale are as follows: renal transplants, 100; liver transplants, 165; heart transplants, 125; heart–lung transplants, 145. Transplant programs consume ~15% of the total laboratory budget, and cost ~$2–2.5 million a year.

Transplantation-Specific Testing

Tissue Typing

The best graft survival outcomes are associated with individuals who receive organs from an HLA-identical sibling, followed by those who receive organs from living, related donors who are haplo-identical. However, controversy persist regarding the impact of HLA A, B, and DR donor–recipient matching on outcome (4–6).

Although some large-scale studies indicate a significant benefit in graft survival for recipients of well-matched grafts, others do not (4–6). Nevertheless, most transplant centers routinely match donor–recipient HLA A, B, and DR antigens, which necessitates the use of a tissue-typing laboratory. It is now common for laboratories to screen for up to 90 antigens by either microlymphocytotoxicity or DNA-based techniques involving polymerase chain reaction technology. At the University of Alberta Hospitals, the following antigens are routinely screened for: HLA A20, B42, C10, and DR17. In addition, the sera from recipients are also tested for panel-reactive antibodies against a large member donor panel representing HLA A, B, and C antigen specificities commensurate with the frequency of these antigens in the donor population.

Markers of Graft Viability/Rejection

One of the major roles of the clinical laboratory is to provide information on the viability of the transplanted graft. In the case of renal transplants, compromised renal function as evidenced by increasing creatinine concentrations can be due to either rejection or CsA-induced nephrotoxicity (7). A number of biochemical and immunological markers have been evaluated to differentiate between the two, including γ-interferon, neopterin, interleukin 2 (IL-2), and IL-2 receptor (8–13). An ideal marker for rejection should be sensitive and able to detect rejection before the onset of clinical symptoms. It should also be specific for rejection. Results of the test should be made available in a clinically relevant time. To date, none of these markers has met these criteria. All markers investigated exhibit an increased concentration upon activation of the immune system, which can occur as a result of rejection or infection (8–12). In addition, the analytical procedures for many of these

Fig. 1. Distribution of testing among laboratory sections for transplant programs at University of Alberta Hospitals. Transplantation-related testing constitutes 15% of total laboratory workload.
analytes are not amenable to the rapid turnaround time required. An exception to the latter was an automated assay developed for the measurement of IL-2 receptor on the Abbott IMx (Abbott Diagnostics, Abbott Park, IL) (12). Although this assay could provide rapid turnaround time in ≤1 h, the clinical usefulness of IL-2 receptor assay in the diagnosis of rejection was not confirmed in multicenter trials (11–13), and Abbott has recently discontinued development of the product.

Biochemical tests have also been developed for the assessment of graft quality prior to harvesting. One such procedure involves the measurement of serum monoethylglycinexylidide (MEGX), a metabolite of lidocaine, in the assessment of donor suitability in liver transplantation (14–17). Lidocaine is metabolized to MEGX via the cytochrome P450 system and has been used as a probe of liver function (14–17). Clinical studies have recently suggested that the kinetics of formation of MEGX after bolus administration would constitute a reliable indicator of intrinsic hepatic function in a donor prior to harvesting, allowing prediction of early posttransplant functional indicators as well as those of late graft survival. Serum concentrations of the MEGX are measured 15 min after an intravenous bolus. A rapid automated assay for MEGX has been developed by using fluorescence polarization technology (FPIA) on the Abbott TDx. Some studies have shown that graft survival was significantly higher when donors had MEGX values >80 µg/L (14, 15). However, other studies have found no relation between MEGX and early functional indicators or graft outcome (16, 17). Further work is required in this area to resolve this discrepancy.

Since all the markers evaluated to date for assessment of graft viability/rejection do not meet sensitivity and specificity requirements for routine use in assessment of transplant patients, we can conclude that the provision of such assays by the laboratory is not a necessary requirement for support of transplant programs.

Immunosuppressive Drugs

One of the major areas for laboratory support of transplant programs is in the provision of a therapeutic drug monitoring program for immunosuppressive drugs. Historically this has involved only the measurement of CsA and in some centers OKT3. However, a number of new immunosuppressive drugs, such as FK506, CsG, RS-61443, RAPA, brequinar, and deoxyspergualin, are undergoing clinical trials. It is anticipated that many of these drugs will receive Food and Drug Administration (FDA) approval within the next few years. Because the majority of these drugs will require monitoring of blood concentrations to maximize immunosuppressive efficacy while minimizing side effects, the laboratory will be faced with new challenges in establishing monitoring programs for these drugs.

CsA. The monitoring of CsA for most laboratories constitutes the major transplantation-specific test. At the University of Alberta Hospitals, CsA is the most frequently monitored drug, with ~12,000 patients' specimens being analyzed for CsA each year at a total cost of $150,000/year. The number of analyses is increasing steadily in proportion to the number of transplant patients. The majority of centers now offer CsA monitoring on either a 6- or 7-day-a-week basis, with results made available the same working day. We can assume that such a frequency in monitoring will be required for other immunosuppressive drugs, as discussed below, to facilitate the clinical management of patients.

CsA exhibits a temperature-dependent distribution between plasma and erythrocytes, with a plasma-to-whole-blood ratio of 0.6 at 37°C (18). The drug is also extensively metabolized, with ~20 metabolites having been isolated and identified (19). The metabolites possess minimal immunosuppressive activity (<10% of that of the parent drug), but they may play a role in CsA-induced toxicity (19). The concentration of the metabolites in blood and tissues exceeds that of the parent drug (19). In instances in which metabolite/parent drug ratios are observed, the measurement of the biologically active metabolites may be warranted; however, to date, this can only be accomplished by HPLC (19). On the basis of these properties of CsA, several consensus guidelines (20–22) have been established for monitoring the drug (Table 3). These guidelines indicate that CsA should be monitored in whole blood with methods that are selective for the parent drug. Of centers participat-

Table 3. Consensus guidelines for monitoring CsA and their applicability for other immunosuppressive drugs.

<table>
<thead>
<tr>
<th>Guidelines</th>
<th>CsA</th>
<th>CsG</th>
<th>FK506</th>
<th>OKT3</th>
<th>RAPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-blood matrix</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Selective assay</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Trough level measurement</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Constant food intake in relation to dosing</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Maximum frequency of monitoring once every 24 h</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Dosing information submitted with request for determination</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinically important metabolites should be measured by a specific method</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Relevant target range based on acceptable clinical criteria</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Same-day turnaround</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Performance characteristics meet acceptance clinical lab practice</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Shipment of specimens at ambient temperatures</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Participation in external quality assurance program</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
ing in an International Quality Assessment Scheme, >90% use whole blood as the medium for analysis, with 89% of the participants using a method that is selective for the parent drug (23, 24). A survey of all Canadian centers performing CsA analysis indicates close to 100% compliance with these two guidelines. Data are not available to assess the compliance with the remaining guidelines for monitoring of CsA, but we can assume that they are also being followed since many of them are consistent with good laboratory practice.

There are four procedures available for the selective monitoring of CsA in whole blood: HPLC; RIA (Cyclotrac SP; Incstar Corp., Stillwater, MN); FPIA (Abbott); and Emit® (Syva, Palo Alto, CA). Of laboratories performing CsA analyses, ~50% use the FPIA procedure, based on information obtained from the British, Canadian, and College of American Pathologists Quality Assessment Programs. Approximately 30–35% of the laboratories use RIA, 10–15% use Emit, and the remaining laboratories (5%) use HPLC for the method of analyses (23). The ranking of these assays with respect to specificity for the parent drug, accuracy, and precision is shown in Table 4 (22, 23, 25–28). The HPLC assay, the most labor intensive, is the most specific for the parent drug; biases due to cross-reactivity with metabolites (shown in parentheses) vary among the immunoassays. In contrast, the FPIA procedure is the most precise (26, 27). With respect to accuracy, the HPLC, FPIA, and Emit procedures compare favorably. A positive bias, related to standardization problems, has been observed with the RIA procedure and has been acknowledged by the manufacturer (23). The Emit assay has the narrowest working range, with concentrations >500 μg/L having to be diluted, whereas in the FPIA dilution is not required until concentrations exceed 1500 μg/L (26–28). The advantage of the Emit assay is the flexibility of instrumentation on which the assay can be performed. The assay is amenable for automation on a number of routine automated analyzers found in clinical chemistry laboratories, thus obviating the requirement for purchasing specialized equipment.

Unlike some other drugs, there is no set concentration range for CsA at which the drug exhibits optimal immunosuppressive efficacy with a low incidence of side effects. We can conclude that as the concentration of the drug increases there is a proportional increase in the incidence of drug-induced side effects (7). This has led to variation in the target concentration of the drug from center to center. However, most centers use ranges that have been established for HPLC even when immunoas-

says that exhibit a positive bias are used. In routine clinical practice the magnitude of this bias in most instances does not affect clinical management.

CsG. CsG is an analog of CsA in which norvaline replaces α-aminoisobutyric acid in position two of the molecule (29). In preliminary studies CsG has shown an immunosuppressive potency similar to that of CsA but less nephrotoxic (30). Multicenter phase 3 trials with the drug are presently underway. As part of these trials the therapeutic monitoring of CsA is required for appropriate dosage adjustment. CsG exhibits a similar temperature-dependent distribution and is metabolized in a manner similar to that of CsA (31). One would therefore expect that the guidelines established for monitoring CsA would be applicable for monitoring CsG. We have assumed that the drug should be monitored in whole blood with assays specific for the parent drug. HPLC methods for measuring the drug, which satisfy the latter requirements, have been reported (32, 33). The FPIA and RIA available for monitoring of CsA also exhibit cross-reactivities of close to 100% and 117%, respectively, with CsG, whereas that of the Emit assay exhibits a cross-reactivity of <2% (34). The cross-reactivities of CsG metabolites in these former two assays are similar to those reported for CsA metabolites (28). However, the steady-state concentrations of CsG metabolites differ from those of CsA, with the secondary metabolites being present at higher concentrations than the primary metabolites, the opposite of the situation with CsA (19, 32).

Most centers involved in monitoring CsG use either the FPIA or RIA procedure. Published reports from our laboratory and others indicate a bias of ~20–30% for FPIA and 30–40% for RIA vs HPLC (33, 34). This is slightly higher than the biases observed for CsA with these assays. This has been confirmed in a quality assurance program for CsG run out of my laboratory for the 34 centers participating in the phase 3 trials. However, if the FPIA and RIA were calibrated with CsG rather than with CsA standards as is the present case, one would expect that the bias with the latter procedure would be reduced by 17% because of the cross-reactivity of CsG in this assay (34). This needs to be confirmed in clinical trials. The therapeutic range for CsG has not been well defined, but the target values are ~25% higher than those being used for CsA in renal transplant recipients. More information is still required to assess the impact of using methods originally established for CsA in the monitoring of CsG; however, this appears to be the most realistic approach at present.

**FK506.** FK506 has undergone extensive phase 3 trials over the past few years and has received FDA approval. The drug has been reported to be 10–100 times more immunosuppressive than CsA; however, the mechanism of action and side effects of FK506 are similar to those reported for CsA (35, 36). From a clinical perspective FK506 does not have a significant advantage over CsA (35, 36). Like CsA, the drug has a temperature-dependent distribution in blood, with most of the drug being sequestered within the erythrocytes (37).

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**Table 4. Ranking of CsA assays for analytical performance.**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specificity for parent drug</th>
<th>Precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>FPIA</td>
<td>(20–25%)*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RIA</td>
<td>(15–20%)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Emit</td>
<td>(3–7%)</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Mean bias in relation to HPLC is shown in parentheses.*
The drug has a narrow therapeutic window (38); thus, monitoring in blood or plasma is required for any patient receiving FK506. However, no guidelines have been established for monitoring this drug. Many of the difficulties originally encountered in the monitoring of CsA are reappearing with the use of FK506 (39). Because of its similarities to CsA in blood distribution, a starting point for establishing guidelines for monitoring FK506 could be based on those established for CsA, as shown in Table 3. Although whole blood now appears to be the preferred medium for measurement of FK506 (23), much of the earlier literature involved measurement of the drug in plasma (38, 39). The assays should also be selective for the parent drug because of the limited immunosuppressive activity of metabolites ascribed to date (40, 41). Consensus on the other guidelines as listed in Table 3 still needs to be resolved. The therapeutic ranges for the drug in whole blood and plasma are 5-20 and 0.5-2.0 μg/L, respectively (42, 43).

Several assays have been developed for measuring FK506 in whole blood, including a HPLC–mass spectrometry assay, an ELISA, a fluorescent particle capture immunoassay developed on the Abbott IMx, and a radioreceptor assay (44-47). All methods require extraction of the drug from whole blood. Results with the ELISA can vary depending on the extraction procedure used (44). In general, the IMx procedure gives values that are ~10% lower than those of the ELISA, which is thought to be due to increased specificity of the antibody used (46). The IMx assay is the most precise of all the assays, with a between-run CV <10% (46, 48). Results with this procedure are usually available within 20 min, compared with 6-36 h for the ELISA (46, 48). The IMx assay, which at present has a lower limit of detection of 5 μg/L, is being reformulated to improve the sensitivity.

OKT3. OKT3 is a murine monoclonal antibody directed against the CD3 receptor on human T lymphocytes (40). It is effective in the treatment and prophylaxis of allograft rejection after solid organ transplantation (49, 50). Dosage adjustments of this monoclonal antibody have been based on the development of anti-OKT3 antibodies or on flow-cytometric determination of CD3+ cells in the peripheral blood (51). Numerous data indicate that monitoring OKT3 serum concentrations may result in more efficacious use of this agent (52, 53). The drug is usually administered at a dose of 5 mg/kg per day for 10-14 days; however, more recently, a lower dose of 2.5 mg/kg per day is being used. At the higher dose, trough concentrations of 800 μg/L should be achieved to attain maximal efficacy (52, 54). However, a number of factors can influence the concentration of the drug, including treatment regimens (prophylactic vs rejection), type of transplant, age of patient, and presence in the patient of anti-OKT3 antibodies (52-54).

OKT3 concentrations have usually been monitored by in-house ELISA procedures (52-54). Screening for the presence of isotypic antibodies to OKT3 can be performed with in-house procedures or with a commercially available ELISA (Sangstat Medical Corp., Menlo Park, CA) (52-55). The latter test involves a plastic cartridge containing four reactive miniwells: a positive reference, a negative reference, the OKT3 test miniwell, and an irrelevant mouse IgG2a miniwell (55). This test allows a rapid detection of IgG antibodies to OKT3, an estimate of the titer, and knowledge of the specificity (antidiotypic and (or) antiisotype (55).

The need for routine monitoring of OKT3 in making appropriate dosage adjustments requires more investigation. Many centers are routinely using the drug without having therapeutic monitoring protocols in place. However, many such centers that monitor the presence of CD3-positive lymphocytes as an indicator of the efficacy of OKT3 treatment may find that establishing methods for measuring OKT3 in place of CD3 is more cost-effective. In patients who require retreatment with OKT3, it is important to know the antibody status. Patients with undetectable or low-titer (<1:100) anti-OKT3 antibodies have retreatment success similar to that of patients undergoing primary treatment with OKT3. In contrast, patients with high-titer (≥1:100) antibodies should be treated with immunosuppressive agents other than OKT3.

RAPA. RAPA is a new immunosuppressive drug with a potency similar to that of FK506 and 100 times greater than CsA. RAPA has been shown to be efficacious in the prolongation of allograft survival in a number of animal models (56). Phase 2 trials in humans are underway. In contrast to other immunosuppressive drugs, detailed therapeutic range studies in animals were performed before initiation of clinical trials (57). Initial work in our laboratory involved investigation of the distribution of the drug in human and animal blood to determine which medium would be the most suitable for analysis (58). Investigation with radioisotopic procedures indicated that RAPA was primarily (95%) sequestered within erythrocytes. The distribution was not associated with any significant temperature dependence (58). This suggested that, for analytical considerations, whole blood would be the preferred medium of analysis. On the basis of these findings, an HPLC method was established for measurement of RAPA in whole blood (59). Pharmacokinetic studies in rabbits indicated that when the drug was administered at immunosuppressive doses, it had a relatively long half-life, with 24-h trough concentrations within the analytical range of HPLC (57, 60). A proportionality between trough concentrations and dose was also observed. The next phase of investigation with RAPA was to relate blood concentrations of the drug to its immunosuppressive efficacy and toxicity in vivo. With a rabbit heart allograft and a canine islet allograft model, trough concentrations >10 μg/L were required for prolongation of graft survival, thus providing some guidelines for therapeutic range (61, 62).

On the basis of the above information, it is most likely that monitoring of RAPA will be required when the drug is used clinically. We hope that the establishment of a preliminary protocol for therapeutic monitoring of RAPA will facilitate its evaluation in clinical studies presently underway. With this approach, many of the problems associated with the establishment of monitor-
ing protocols for other immunosuppressive drugs such as FK506 may be avoided (63).

Other immunosuppressive drugs. A number of other immunosuppressive drugs are in various stages of clinical trials. One such drug is RS-61443, the morpholinoethyl ester of mycophenolic acid (MPA). The immunosuppressive activity of RS-61443 is expressed only after hydrolysis to MPA upon absorption. Therefore, RS-61443 can be considered to be a prodrug (64). It is an inhibitor of purine synthesis and therefore may replace azathioprine in immunosuppressive dosage regimens (64). HPLC methods have been described for measurement of the drug in plasma; concentrations associated with therapeutic efficacy are most likely to be in the milligrams per liter rather than the micrograms per liter range (65). An Emit method for measurement of MPA is presently undergoing development. The drug is relatively innocuous, with few side effects reported (64, 66). The therapeutic monitoring of the drug has not been included as part of the clinical trials. However, the bioavailability of the drug has been shown to change as time from transplantation increases (66). In addition, drug–drug interactions, which have been shown to alter the pharmacokinetics, have been reported (66). Further investigation is required to determine whether the routine monitoring of MPA will be required.

Brequinar, an inhibitor of pyrimidine synthesis, is now undergoing phase 3 trials. An HPLC method for analysis for this drug has been reported (67). Peak concentrations >2 mg/L in animal studies have been shown to be associated with optimal immunosuppressive efficacy (68). Deoxyxergualalin is another potent immunosuppressant whose mechanism of action has not been completely elucidated (69, 70). Trough concentrations of the drug in the milligrams per liter range are required for optimal immunosuppressive efficacy (70). It is not known whether the routine monitoring of these drugs will be required.

Future Prospects

Because of a lack of donors, increased emphasis will be placed in the future on the use of animals as donors for transplantation (xenotransplantation). The reference ranges for a number of chemical and hematological constituents in blood of animals differ from those of humans. In a recent report on hepatic xenotransplantation with a baboon donor, the biochemical profile of the constituents synthesized in the liver after transplantation was more similar to that of the baboon than of man (71). This suggests that laboratories will have to establish the appropriate analytical procedures and reference ranges to accurately monitor recipients of xenografts.

The drugs discussed here will be used not alone but in various combinations with other immunosuppressive drugs. Although guidelines for monitoring many of these drugs have not been established, the guidelines previously established for CSA may be relevant. The applicability of these for some new immunosuppressive drugs is shown in Table 3.

Additional major challenges for clinical laboratories in the future will be the establishment of new procedures for the assessment of graft availability/rejection as well as the implementation of therapeutic drug monitoring programs for these new immunosuppressive drugs. To facilitate the latter, the following areas should be considered. Since many of the drugs will be used in combination, simultaneous assays that measure more than one drug should be developed. There should be flexibility in the type of instrumentation available for assay performance. Ideally, the assays should be automated on the routine analyzers available in clinical laboratories. When whole blood is the matrix for analysis, the methods should not require specimen pretreatment. Methods in which the drugs can be analyzed in dried blood spots should be developed, which would obviate the need for patients to go to a clinic for procurement of specimens. Point-of-care methods for analysis of immunosuppressive drugs should be developed to permit analysis of concentrations in the clinic, thus permitting dosage adjustments to be made in a shorter timeframe. Furthermore, investigations into the usefulness of monitoring the biological effect of the drugs rather than the drug concentrations are required. Development in many of these areas is under way.

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