Sensitization by previous pregnancies or transplants is considered unavoidable, but it is transfusions given to these patients that leads most often to broad sensitization. Both leukocytes and red cells carry a significant HLA antigen load, and residual leukocytes and/or red cell HLA may explain why leukocyte-reduced units are unable to prevent sensitization to any significant degree. Prevention of sensitization will require a more active effort to avoid blood transfusions, whenever possible. When transfusions are required, there is evidence that the use of HLA-matched blood or immunosuppression in selected situations may reduce sensitization, even in patients previously exposed to alloantigens. These additional measures are not logistically straightforward or devoid of risks and need to be confirmed by rigorous studies. However, remaining as passive observers when patients become broadly sensitized should no longer be considered an acceptable alternative for potential transplant recipients.

Key words: HLA antibodies, organ transplants, sensitization, transfusions

Abbreviations: DST, donor specific transfusions; ESA, erythropoietin-stimulating agents.

Received 01 March 2011, revised 26 May 2011 and accepted for publication 25 June 2011

Sensitization continues to be a serious and difficult problem for organ transplant patients. Although the use of plasma exchanges and immunosuppression to transplant sensitized patients has a long history (1), only in recent years desensitization has been brought to the forefront of organ transplantation. The hallmark of these recent, more aggressive approaches to transplant sensitized patients has been a “can do” attitude, whereby expensive and cumbersome treatments with uncertain rewards (2) were no longer an impediment to offer these patients the option of transplantation. Clearly, a similar effort has not been applied to the prevention of sensitization.

Although sensitization is produced by previous transplants, pregnancies or transfusions, the incidence, degree and duration of the antibody response (and therefore its clinical significance) are the result of complex interactions largely determined by the nature of the alloantigenic event and the immunologic history of the patient. Unfortunately, this complexity is often overlooked in favor of misconceptions, like erythrocytes do not express HLA antigens, transfusions induce only HLA antibodies against the transfusion donor or leukocyte-reduced blood is the solution to transfusion-induced sensitization.

The goals of this discussion are to revisit the immunological effects of blood transfusions, to discuss opportunities to prevent sensitization and to energize transplant professionals in implementing prevention measures that can be effective in a significant number of patients.

HLA Molecules in Blood Transfusions

**HLA content of leukocytes**

Much of the antigen load in a blood unit is present in lymphocytes, which express between $10^5$ and $2 \times 10^5$ class I HLA molecules per cell (3). Other leukocytes express less (4). Platelets are rich in HLA molecules, but they are routinely removed from packed red cell units. Monocytes also express class II HLA antigens ($1–2 \times 10^4$ antibody–phycoerythrin molecules/monocyte) (5).

**HLA content of erythrocytes**

The HLA class I molecules in erythrocytes are expressed constitutively at low levels, and at higher levels under genetic and environmental influences. Radiolabeling techniques and Scatchard analysis show that erythrocytes contain 100–2000 class I HLA molecules per cell (6). Thus, the HLA content of erythrocytes is as significant as that of leukocytes because there are 1000 times more red cells than leukocytes in a blood unit. Erythrocytes from individuals positive for HLA-B7 express more, a fact recognized long ago as responsible for the presence of the red cell antigen Bg<sup>a</sup> (7). Erythrocyte HLA expression is also higher in patients receiving interferon alpha for viral hepatitis and in HIV infection (8).
Scornik and Meier-Kriesche

Down-Regulatory Effects of Blood Transfusions

Despite the high HLA content of blood transfusions or perhaps because of it, transfusions do not often elicit an antibody response in naive recipients. In fact, they are immune down-regulatory, as shown by the “transfusion effect” of improved renal graft survival when patients received either random donor (9) or donor-specific (10) transfusions (DSTs). Multiple clinical and experimental studies have implicated induction of anergy, inhibitory cytokines, regulatory T cells and other mechanisms triggered by the intravenous administration of alloantigens, as mechanisms involved in this phenomenon (11). The immune modulating effect of transfusions seems to be also responsible for an increased rate of infections in patients with trauma (12) or burns (13). Improved transplant outcomes were seen in transfused patients during the 1970s and 1980s, when 1-year renal graft survival was 60–70%. In the modern immunosuppression era, with 1-year graft survival rates around 90%, this effect is less evident, possibly still present according to some reports (14) but not according to others (15).

Sensitization by Blood Transfusions

The risk of sensitization by transfusions has to be evaluated in the context of the immunologic history of the patient, as they are down-regulatory in naive recipients and stimulatory in patients previously exposed to alloantigens. Table 1 summarizes these interactions, which are described below in more detail.

Table 1: Immunogenicity of blood transfusions, pregnancies and transplants

<table>
<thead>
<tr>
<th>Recipient</th>
<th>%sensitized</th>
<th>CDC</th>
<th>Solid phase</th>
<th>Titer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusions alone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent</td>
<td>10–12</td>
<td>10</td>
<td>Low</td>
<td>16–18</td>
<td></td>
</tr>
<tr>
<td>Distant</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>Medium</td>
<td>20, 21</td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>35</td>
<td>35</td>
<td>Medium-high</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>50 and up</td>
<td>50</td>
<td>Medium-high</td>
<td>24, 25</td>
<td></td>
</tr>
<tr>
<td>Previous pregnancies alone</td>
<td>5</td>
<td>24–33</td>
<td>Low-medium</td>
<td>20, 27, 28</td>
<td></td>
</tr>
<tr>
<td>Plus transfusions</td>
<td>40</td>
<td>52</td>
<td>High</td>
<td>23, 28, 29</td>
<td></td>
</tr>
<tr>
<td>Previous transplants alone</td>
<td>17</td>
<td>72</td>
<td>Low-high</td>
<td>30, 31</td>
<td></td>
</tr>
<tr>
<td>Plus transfusions</td>
<td>60–78</td>
<td></td>
<td>High</td>
<td></td>
<td>6, 32</td>
</tr>
</tbody>
</table>

Sensitization by transfusions as the sole alloimmunogenic event

As mentioned above, transfusions are poorly immunogenic in naive recipients. The incidence of HLA antibodies in renal transplant candidates, who had no previous pregnancies or transplants, is 10–12%, as measured by either cytotoxicity in patients who received up to 20 transfusions (16) or enzyme immunoassay (17,18). When HLA antibodies were produced, they seldom reached high levels and decreased to baseline after a few months, even after subsequent transfusions (16,17). Furthermore, patients who became sensitized by protocol transfusions were successfully transplanted with deceased donor kidneys when antibodies declined (19). The low immunogenicity of blood transfusions is supported by recent studies in male and nulliparous female blood donors that reported sensitization rates of 1.7% in donors who had received transfusions in the past and 1.0% in those who had not (not significant) (20,21). Although these considerations stress that clinically significant sensitization in these patients is uncommon, the potential for sensitization is always present. The practice of transfusing only when it is strictly necessary should be universal, although using prevention measures, such as immunosuppression or HLA matched blood (discussed later) when transfusions are unavoidable, may not have a favorable risk or cost benefit ratio in naive patients.

Sensitization by transfusions according to age

When immune reactivity is measured in different experimental systems, a consistent finding has been that responsiveness is highest in young individuals and decreases with advancing age (22). Along the same lines, blood transfusions induced sensitization in up to 35% of the patients aged 5–20 years as compared to 7.5% of the patients >20 years (17). However, the sensitization effect was temporary in most patients and antibodies returned to baseline levels between 5 and 11 months after the transfusions. Higher sensitization rates were also noted in younger patients, who were transfused at the time of implantation of ventricular assist devices (23).

Sensitization by multiple transfusions

Renal transplant candidates rarely receive multiple or massive transfusions, so that there is little information available in these patients. In heart failure patients undergoing implantation of ventricular assist devices, multiple transfusions are common and their immunogenicity is directly related to the number of units given (24). In patients with sickle cell anemia, 35–50% of them produced lymphocytotoxic antibodies when given 50–100 transfusions and 71% when given >200 (25). Sensitization is also common in patients with hematological malignancies (see below).
In summary, although the risk of sensitization by transfusions is low in naive recipients, sensitization does occur, especially in pediatric recipients and when multiple transfusions are given.

**Contribution of Pregnanacies to the Risk of Sensitization by Transfusions**

**Sensitization by pregnancies alone**

In a study where patients’ sera were tested during pregnancy in a lymphocytotoxic crossmatch against the partner’s cells, antibodies were not detected until about the 28th week of gestation (26). At the time of delivery, the sensitization rate was 32% and declined thereafter. Studies of blood donors with past pregnancies show a 5% sensitization rate by cytotoxicity (27) and 24–33% by solid phase techniques (20), with a stepwise increase with higher number of pregnancies. Similar results were obtained in renal transplant candidates with past pregnancies and no transfusions (28).

**Sensitization by transfusions in patients with past pregnancies**

In renal transplant candidates who had previous pregnancies, the induction of HLA antibodies by transfusions is more frequent and of higher titer: 5% of patients with no transfusions, had cytotoxic antibodies with a modest titer (panel-reactive antibody [PRA] <50%), whereas in those transfused, the incidence rose to 40% and the panel reactivity was >50% in 14% of them (28,29). Results were similar in heart failure patients who received a ventricular assist device (23). An interesting point is that susceptibility for broad sensitization by transfusions is higher in multiparous patients who already show low-level antibodies, as detected by more sensitive techniques (28).

In summary, most patients with past pregnancies and no transfusions are either weakly or not sensitized, and usually proceed to transplantation with no undue delays. In contrast, it is not uncommon to see multiparous patients with little or no HLA antibodies at presentation, who become highly sensitized by a few transfusions before or during listing.

**Sensitization by Previous Transplants and by Previous Transplants Followed by Transfusions**

**Sensitization with a functioning graft**

Patients, who have a functioning graft, may become sensitized while they are under immunosuppression. De novo posttransplant antibodies are a risk factor for graft rejection and graft loss, although they have also been observed in patients with stable renal function reviewed in Ref. 30. De novo antibodies are rare early after transplantation, become more frequent with time after transplant and can be a major factor in late graft failure. Although some patients appear to make antibodies through an escape from immunosuppression, decreased immunosuppression due to noncompliance or presence of infections or posttransplant lymphoproliferative disease probably play a major role in sensitization.

**Sensitization after transplant loss**

The incidence of sensitization in patients who lost a transplant reported in various studies is around 70%. However, most patients are not sensitized at the time of transplant loss. In a recent study at our center, of 85 patients followed after transplant loss, only 16 (19%) were sensitized at the time of graft loss (31). About half of the remaining patients made antibodies subsequently, possibly because of discontinuation of immunosuppression, transfusions or graft nephrectomy, for an overall rate of sensitization of 70%. Interestingly, similar rates (73%) were seen in patients who lost the graft immediately after transplant, due to vascular complications (31). A factor to take into account is HLA matching of the first transplant. In a retrospective study from the Scientific Transplant Registry, HLA-A, -B mismatch increased the risk of sensitization after graft loss, suggesting that HLA-A, -B matching can contribute to reducing sensitization after transplant loss (32).

Another interesting observation was reported in two unsensitized retransplant candidates. After one or two transfusions, both patients made antibodies that were specific for the previous transplant donor but not for the blood donors, as shown by a negative crossmatch against blood donor lymphocytes (33). This observation and the fact that the majority of previously transplanted patients, most of them also transfused, show antibody specificity for the antigens of the transplant donor, suggests that in these cases, the transplant HLA antigens are the source of antigenic stimulation and that the transfusions represent a triggering event, either by HLA cross-reactivity or nonspecific polyclonal B-cell activation.

Thus, patients who remain unsensitized at the time of transplant loss may still be at high risk of making broadly reactive antibodies if transfused. This point is critical for applying measures to minimize sensitization when retransplantation is an option.

**Effect of Leukocyte Depletion on Sensitization by Red Cell Transfusions**

**Hematological malignancies**

This population includes patients with leukemia and related conditions, who undergo intensive chemotherapy and receive multiple blood products to support them during the pancytopenic period. These patients may develop HLA antibodies and become refractory to further platelet transfusions. This is a significant problem and multiple studies have addressed the effectiveness of leukocyte-reduced
red cell and platelet units (<5 × 10⁶ leukocytes/unit) to reduce allosensitization. Most studies reported a benefit of leukocyte-reduced platelet transfusions, the most quoted being the NIH-sponsored trial to reduce alloimmunization to platelets (TRAP) (34). The incidence of sensitization decreased from 45% to 18% by leukocyte reduction, a finding that also applied to patients with previous pregnancies. However, the TRAP investigators cited other studies that reported no effect in multiparous patients, leading them to conclude that this issue requires further research. This landmark study has been the source of some confusion when applying the results to the organ transplant setting, because it only investigated the effect of leukocyte-reduced platelet units, not red cell units (leukocyte-reduced red cells were given to patients in all study arms). In addition, these patients have a fundamentally different primary disease course compared to organ transplant candidates. Patients undergoing intensive chemotherapy to induce disease remission, receive multiple red cell and platelet products in a short period of time whereas in the transplant setting, transfusions can predate the transplant by many years and are usually few and isolated events. Therefore, extrapolation of results from one population to the other has limited value.

Transplant candidates

In fact, several publications report that leukocyte reduction is not helpful in organ transplant candidates. In two observational studies with a few high risk patients, blood that was leukocyte-reduced (35) or leukocyte-reduced and selected for low content of red cell HLA (6) consistently induced sensitization similar to nonreduced units. In a randomized prospective study, leukocyte-reduced red cells provided no benefit when compared with nonreduced packed red cells in terms of sensitization and the likelihood of receiving a kidney transplant or eventual kidney graft survival (36). The effect of leukocyte-reduced transfusions in kidney transplant candidates was also evaluated in two cohorts that received transfusions before and after the implementation of universal leukoreduction. Leukocyte-reduced transfusions did not confer any protection against allosensitization (18). Finally, surgery patients who were randomly assigned to receive transfusions that were leukocyte-reduced before or after storage or not leukocyte-reduced, resulted in similar posttransfusion allosensitization frequencies (37,38).

Thus, leukocyte-reduced red cell transfusions are still immunogenic in transplant candidates, a fact probably involving HLA molecules still present on residual leukocytes and possibly also on erythrocytes. Senescent erythrocytes are removed from the circulation during the weeks after the transfusion, providing a steady supply of foreign HLA antigens to antigen presenting cells. This process can conceivably contribute to initiate or, more likely, recall a T-cell response through the indirect pathway of allostimulation.

Educational Programs for Sensitization Prevention

Although most sensitized patients had past pregnancies or transplants, the previous discussion highlights the point that high sensitization occurs almost always when blood transfusions are given after pregnancies or graft loss. Sensitization may not be avoided in all cases, but there exists the potential to reduce its incidence by applying a range of measures that target patients in specific situations (Figure 1).

Raising the profile of sensitization as a significant clinical problem

It is critical to think about sensitization before ordering transfusions to transplant candidates. Although management of the sensitized patient is a frequent theme of educational activities, prevention is seldom discussed. Its inclusion could raise the awareness of the problem, encourage prevention measures and perhaps spare transfusions in patients who could do without.

Identifying patients at risk

Because of the higher risk of patients with previous pregnancies or transplants, especially if they are already weakly sensitized (28), transplant teams should have protocols for identifying them and communicating with patients and their referring physicians about sensitization risk, in case transfusions are needed. Cardiac surgery in heart transplant candidates is also a risk factor, especially in children, because of the possibility of many transfusions in a short period of time.

Avoiding transfusions

The use of blood transfusions in renal disease patients decreased between 1992 and 2005, but it continues to

Figure 1: Strategies for prevention of sensitization.
be common (39). The decrease can be attributed to the increased use of erythropoietin stimulating agents (ESAs) and increased awareness of transmission of infections and other untoward effects. The ESAs have risks (40) that need to be balanced against the consequences of sensitization. Blood banks can advise regarding the collection of one or two blood units from the patient for autologous transfusions during prospective elective surgery.

Another factor worth mentioning is the need for transfusions in hospitalized patients due to multiple blood drawings for lab tests. Program directors should act aggressively to avoid this unnecessary risk of sensitization.

**Strategies for Prevention of Sensitization When Patients Need Blood**

Table 2 summarizes some clinical scenarios where there are opportunities to minimize sensitization. When patients need to be transfused, using HLA-matched blood or immunosuppression, are the options to be considered.

**HLA-matched transfusions**

During the era of DSTs, patients were given blood from their prospective transplant donors, typically three reduced volume transfusions during a period of 1–2 months. Under these protocols, many patients were transfused with blood from HLA-typed donors who had a variable degree of HLA match. As expected, when more HLA antigens were matched, there was an independent association with a significantly decreased risk of sensitization (41). The HLA-A and -B matched transfusions were also evaluated for their benefit on transplant outcome. Although the outcome results were controversial, these patients did not produce broadly reactive antibodies. Additional evidence was obtained in 24 patients who had prior pregnancies and were positive for low-level HLA antibodies, that is, were at a very high risk of broad sensitization if transfused. Using the same protocol as for DSTs, only one patient produced additional HLA antibodies (0/14 transfused with 0-mismatched red cells and 1/10 transfused with one or two mismatches) (42). Matching for one HLA-DR antigen appeared to reduce sensitization (43), although further studies are needed to establish the minimal degree of matching to achieve beneficial results.

**Logistic issues**

HLA-matched red cell transfusions would represent a new treatment modality and as such it would require the use of existing resources, the creation of new resources and a stringent patient selection. In terms of existing resources, HLA class I-matched platelet transfusions are routinely used for patients who become refractory to random donor platelets due to HLA sensitization. Some blood centers perform HLA-typing of donors who are willing to undergo plateletpheresis on a regular basis. Other blood centers recruit donors for the National Marrow Donor Program where the donors are HLA-typed. In this case, individual blood centers can retrieve the HLA types and use these donors for the needs of their communities. Blood centers communicate with each other directly or indirectly through clearinghouse organizations, expanding the donor pool and the possibility of finding appropriately matched donors. This existing resource would represent the creation of a new resource, if the demand for HLA-matched donors encourages the development of pooled databases that can facilitate identifying matched donors or even units already existing in the combined inventory. Finally, HLA-matched blood should probably be limited, at least at first, to patients at high risk of sensitization when transfused (multiparous, retransplant candidates and sometimes pediatric patients), to allow for a gradual transition to offering this new blood product by blood providers.

Most HLA-matched units can be available within 48–72 h, so that possible indications include preparation for elective surgery or symptomatic anemia requiring more prompt red cell replacement than that achieved with ESAs, iron, etc. HLA matching will add to the cost of a blood unit, which will vary at different places. Transfusion induced graft versus host disease is a possible complication of HLA-matched blood transfusions, and blood banking regulations require irradiation of the units to avoid it.

| Table 2: Measures that can reduce sensitization when patients need red cell transfusions |
|-----------------------------------------------|-----------------------------------------------|
| Clinical scenario                                      | Potential interventions                      |
| Adults with no previous pregnancies or transplants    | No prevention measures needed (2B)¹           |
| Children                                              | HLA-matched blood (2B)                        |
| Anemia                                                | Prevent need for transfusions with ESAs, iron, other supplements (1B) |
| Transfusion during elective surgery in patients with past pregnancies or transplants | HLA-matched blood (2B) or short course of immunosuppression (4) if transfusion is unavoidable |
| Graft nephrectomy                                      | HLA-matched blood (2B)                        |
| Progressive graft dysfunction with impending retransplant | Short course of immunosuppression (4)        |
|                                                      | Prolong immunosuppression (4)                 |
|                                                      | HLA-matched blood (2B)                        |
|                                                      | Prolong immunosuppression (4)                 |

¹Levels of evidence: 1A, systematic review of randomized, controlled trials (RCTs); 1B, RCTs with narrow confidence interval; 1C, all or none case series; 2A, systematic review cohort studies; 2B, cohort study/low quality RCT; 2C, outcomes research; 3A, systematic review of case-controlled studies; 3B, case-controlled study; 4, case series, poor cohort case controlled; 5, expert opinion.
Procurings HLA-matched blood will have to involve interactions among specialists in the histocompatibility labs, transfusion services and blood centers. Because HLA-matched red cell units are not a product offered at the present time, the logistic obstacles will be considerable. However, it is pertinent to remember that in 1980s, clinicians and blood centers were in a similar situation trying to find matched unrelated stem cell transplant donors, which is the precedent for the international system now in place that is part of the routine care for those patients.

**Immunosuppression in patients who need blood transfusions**

There is evidence suggesting that immunosuppression may reduce or prevent sensitization by transfusions. During the DST era, administration of azathioprine (44) or cyclosporine (45) for 3–4 weeks seemed to reduce the risk of sensitization. Also, blood transfusions given to patients with a functioning graft, and therefore receiving maintenance immunosuppression, were not associated with the production of HLA antibodies (46). Thus, immunosuppression is another logical modality that could be used in specific circumstances, although further studies will be needed to establish clear indications and duration of treatment according to the clinical situations of individual patients.

A possible scenario for confirmatory studies is patients who lost graft function, either immediately after graft thrombosis or after chronic graft dysfunction, and need to undergo transplant nephrectomy. Because many of these patients receive transfusions and become sensitized, prolonging immunosuppression until after surgery and patient recovery may be a means of decreasing sensitization rates. Even patients not needing graft nephrectomy may benefit of not discontinuing immunosuppression, if they have a live donor available or are otherwise expected to be retransplanted in a short period of time. Another possible scenario is patients, who need a limited number of transfusions without delay, and remain as transplant candidates.

**Conclusions**

Existing evidence supports the concept that sensitization of transplant candidates can be minimized under certain circumstances. Clinicians should not order blood transfusions without thinking about sensitization and its possible consequences. Surgeons should not schedule elective surgery without exploring all possibilities to avoid sensitization in case the patient needs blood. Transplant centers should develop protocols to identify patients at risk, discuss the possibility of sensitization with the patient and referring physicians, help referring physicians when patients need blood and implement procedures to avoid sensitization, whenever possible. Educational activities by professional societies should emphasize prevention, not just treatment of sensitization.

If transfusions are needed, HLA matching may be used when there is time to procure blood units, and immunosuppression may be used for acute needs. However, there are still many questions that remain to be answered, including what is the minimum degree of matching that can be effective or if one haplotype matched relatives may be used or what are the best strategies to make matching logistically successful. Immunosuppressive protocols (drugs of choice, length and safety of treatment) need to be developed and tested in specific circumstances, such as at the time of graft loss, elective surgery or other situations, when transfusions cannot be avoided. Also, a question for future consideration is whether these measures should be applied only to patients who had previous transplants or pregnancies or be more inclusive. The necessary effort will not be trivial, as it has not been to treat sensitized patients. Although a “can do” attitude helped implement desensitization protocols and understand their potentials and limitations, a similar determined approach will be needed to apply prevention measures and expand its benefits.

**Disclosure**

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. Dr. Scornik is a paid consultant for Amgen Inc.

**References**

Sensitization by Blood Transfusions


34. Slichter SJ; for the trial to reduce allosensitization to Platelets study group: Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. N Engl J Med 1997; 337: 1861–1869.


