REVIEW

Immunomodulation by ultraviolet light: clinical studies and biological effects

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The interest of immunologists in ultraviolet (UV) irradiation stems from observations made in vitro *and* in vivo. In vitro, *UV irradiation inhibits mitogen and mixed lymphocyte culture (MLC) responses and* in vivo, *it can induce cutaneous anergy, apparently via suppressor cells and serum factors. At present much interest is focused on the possible use of UV irradiation to permit transfusion without allosensitization and transplantation without either rejection or graft-versus-host disease (GVHD). Here, Derwood Pamphilon and colleagues discuss the current uses and potential of UV irradiation in transfusion and transplantation and relate these to experimental evidence on its effects at the cellular level.*

The UV region of the electromagnetic spectrum is divided into three portions: UVA (320–400 nm), UVB (290–320 nm) and UVC (200–290 nm). UVC causes erythema of normal skin and has been termed germicidal radiation, since it is effective in killing single-celled organisms. UVA radiation causes melanin pigmentation of skin. Little UVC radiation reaches the earth and UVA has minimal biological activity compared with UVB unless used in conjunction with a chemical photosensitizing agent[1].

The biological effects of UV radiation include damage to lymphocytes. UVC is approximately ten times more effective than UVB and 10^5 times more effective than UVA as measured by trypan blue dye exclusion[2]. These biological effects depend upon the dose as well as the wavelength. The dose is calculated by measuring the intensity of the source using a radiometer. Intensity is a measure of energy reaching a unit area of surface in one second and may be expressed as Watts m^{-2} ($W m^{-2}$). Dosage, given as Joules m^{-2} ($J m^{-2}$), is calculated from the equation:

$$\text{Dosage} = \text{Intensity} \times \text{Time}$$

Clinical importance of UV irradiation

The abolition of alloreactive responses after UV irradiation suggests that it may be beneficial in reducing alloimmunization after platelet transfusion and in prolonging the survival of certain allografts. This might be achieved if lymphocyte or accessory cell functions were abolished, while those of cells such as platelets, haemopoietic stem cells and pancreatic islets were retained.

Platelet transfusions

Alloimmunization to the major histocompatibility complex (MHC) antigens present on both leucocytes and platelets occurs in 50–70% of patients receiving multiple platelet transfusions[3] and is due to contaminating leucocytes; pure platelet suspensions are not immunogenic[4] and may in fact tolerize to subsequent leucocyte-containing platelet transfusions[5]. Leucocyte removal by filtration is currently the method of choice to reduce or prevent alloimmunization but removal is not completely efficient and allosensitization is not entirely eliminated[6].

There is direct evidence that UV irradiation inactivates dendritic cells (DCs) and DCs appear to be of central importance in the induction of allosensitization[7] (*vide infra*): the addition of purified nonirradiated DCs to blood transfusions treated with UV irradiation restores their ability to sensitize canine marrow graft recipients.

The possibility of UV inactivation of leucocytes in platelet concentrates was first reported by Kahn *et al.*[8], who noted that UVB abolished lymphocyte responses in MLC without affecting platelet function[8]. Further studies[9–11] have shown that UVB irradiation permits platelet storage for 5 days with retention of functional characteristics comparable with controls, while the reactivity of contaminating leucocytes in MLC is abolished[9]. Autologous recovery and survival of platelets treated with UV irradiation is not impaired *in vivo*[10,11] even after irradiation with 3000 J m^{-2} and 5 days storage[10] and the incremental rise in platelet count seen in thrombocytopenic recipients is comparable with those obtained with nonirradiated transfusions[11]. Alloimmunization in dogs receiving platelet transfusions was reduced to 8% when these were irradiated with UVC compared with 86% of recipients of control transfusions. Subsequently half of the nonimmunized dogs remained tolerant to non-UVC-irradiated platelets[12]. The early evidence suggests that alloimmunization can be similarly avoided in human platelet transfusions (authors' unpublished observations) and clinical trials are under way.

Transplantation

As UV irradiation abrogates MLC responsiveness, it may prevent both GVH and graft rejection/host-versus-graft (HVG) reactions in clinical transplantation. Smith and Miripol[13] have shown, using a popliteal lymph node

assay, that GVH and HVG responses *in vivo* are lost in mice exposed to a dose of UV radiation that is double that required to abolish the mixed lymphocyte response (MLR) *in vitro*. UV irradiation may be used where (1) direct irradiation of the graft is physically practical, for example bone marrow and pancreatic islets, and (2) irradiation of donor-specific transfusions (DST) prior to transplantation may help to induce specific tolerance to donor tissue.

HVG responses. Transplant patients are vulnerable to a variety of infections due to the immunosuppressive effect of agents given to prevent rejection. In addition, bone marrow transplant (BMT) patients receive myeloablative conditioning regimens that are immunosuppressive. Induction of specific unresponsiveness to donor tissue would reduce the need for immunosuppression. Canine bone marrow allografts from MHC incompatible littermates are accepted when infused after three DST treated with UV irradiation have been given. If the DST are nonirradiated, or nonirradiated DCs are added after UV irradiation, there is uniform graft rejection[7,14].

The effects of UV-irradiated DST in preventing HVG responses and prolonging allograft survival have been investigated in animal models of bone marrow, pancreatic islet and cardiac transplantation (reviewed in Refs 15–17). The effect of direct UV treatment of the graft in pancreatic islet and corneal grafting[15,17] has also been studied and the results of both of these approaches are summarized in Table 1. For successful transplantation in the latter category it is essential that alloreactive responses are abolished, while the function of specialized cells within the graft is preserved. Histoincompatible rat pancreatic islets of Langerhans irradiated with 900 J m^{-2} of UVB and transplanted into diabetic Lewis recipients are permanently accepted as indicated by normalization of blood glucose[18]; injection of nonirradiated DCs into long-term acceptors leads to islet allograft rejection[18].

GVH responses. Bone marrow transplantation carries the risk of GVHD. Bone marrow T cells are inactivated by a dose of UVB of 50–100 J m^{-2}; at this dose human and murine haemopoietic stem cells, measured respectively by *in vitro* clonogenic[19] and spleen colony-forming-unit assays[20], are not significantly affected. Differential sensitivity between T cells and stem cells is not seen with UVC[19]. Studies in our laboratory using 100 J m^{-2} UVB from a different source (mean wavelength 310 nm compared with 302 nm in Ref. 20) show that direct irradiation of BALB/c (H-2^d) mice marrow plus splenocytes allows successful transplantation without GVHD in 50% of CBA (H-2^k) recipients[21]. Surviving mice have donor-type haematopoietic tissue but assays performed *in vitro* and *in vivo* show reduced stem cell proliferation and slow haemopoietic recovery (Ref. 21 and authors' unpublished observations). The same dose, 100 J m^{-2} of UVB, is required to abolish the *in vitro* response to allogeneic cells so that at higher wavelengths there is less differential sensitivity between haemopoietic and lymphoid cells[21].

In rats, only one out of 14 Lewis strain recipients developed GVHD when infused with allogeneic UVB-treated ACI strain marrow and peripheral blood lymphocytes[22]. Adequate preservation of stem cell function was established by acceptance of syngeneic grafts in these rats[22]. UV-treated allogeneic leucocytes did not induce GVHD after syngeneic BMT in mice[19] or autologous BMT in dogs (Table 1)[23].

These studies suggest that UV irradiation may play a valuable role in the prevention of GVHD in human bone marrow transplantation. Established methods of GVHD prophylaxis in BMT are unsatisfactory. Immunosuppressive agents such as cyclosporin, anti-lymphocyte

Table 1. Studies with UV irradiation in animal transplantation models

Species		Transplant model	Effect on Rejection response	Effect on GVH response	Ref.
Mouse	(1)	Allogeneic BMT Parent → F_1 (C57BL/6 × A/J)		Reduced	17
	(2)	Allogeneic BMT BALB/c → CBA		Reduced or abolished	21
	(3)	Allogeneic BMT Parent → F_1 (B6 × D2)		Abolished	20
	(4)	Corneal transplant BALB/c → C57BL/6	Improved graft survival		15
Rat	(1)	UV-irradiated DST then cardiac allograft Lewis → AC1	Permanent graft acceptance		16
	(2)	UV-irradiated pancreatic islet transplant Lewis → AC1	Indefinite prolongation of graft survival		18
	(3)	Allogeneic BMT AC1 → Lewis		Abolished	22
Dog	(1)	UV-irradiated DST then allogeneic BMT	Permanent graft acceptance only if DST irradiated		14
	(2)	Autologous BMT followed by histo-incompatible leucocyte transfusions		Reduced (200 J m^{-2}) Abolished (10 000 J m^{-2})	23

globulin and steroids increase susceptibility to infection and the removal of alloreactive T cells from the graft is associated with increased graft rejection and leukaemic relapse[24]. Although this has not yet been reported in BMT studies, the loss of (1) stimulatory ability in human[9,25] and mouse[13] MLC *in vitro* and (2) of HVG reactivity in mouse popliteal lymph node assays[13] *in vivo* indicate that a bone marrow graft irradiated with UVB may not be recognized as foreign.

There is evidence that bone marrow transplant recipients that develop GVHD are less likely to have a leukaemic relapse, suggesting a graft-versus-leukaemia (GVL) effect[24]. Interestingly, although stable long-term chimerism in rat BMT is observed after graft treatment with UV irradiation, there is evidence of a low grade, clinically-undetectable GVH reaction[22]. Further studies are now required to assess whether a GVL effect might also be detected after UV irradiation–BMT in appropriate animal models.

The clinical studies described above indicate that inactivation of antigen-presenting cells (APCs), for example DCs, is important in inhibiting HVG reactions and allosensitization. By contrast, it is not yet clear whether abolition of GVHD occurs as a direct result of T-cell inactivation or via loss of an intermediary or costimulatory signal provided by APCs. This hypothesis could be evaluated by readdition of specialized leucocyte subpopulations to marrow grafts treated with UV irradiation in animal studies.

Biological effects of UV irradiation

In the following sections data from studies on the cellular effects of UV irradiation *in vitro* and *in vivo* is examined with a view to identifying mechanisms that are relevant to clinical studies.

Inhibition of in vitro *responses to mitogens and alloantigens*

In 1971, Lindahl-Kiessling and Safwenberg[25] reported that lymphocytes exposed briefly to UV irradiation could not stimulate allogeneic cells in MLC or respond to mitogenic stimulation. This observation has been confirmed by others[9,13,26] and does not appear to result from loss of viability. UV irradiation is known to perturb many of the activities associated with normal cellular responses to mitogens and alloantigens (Fig. 1).

Cell clusters and surface ligands. Interaction between specialized APCs, such as DCs, and lymphocytes to form clusters is necessary for lymphocyte activation and is abolished when purified DCs, treated with UV irradiation are cocultured with accessory-cell-depleted lymphocytes in the presence of concanavalin A[27]. The generation of cytotoxic secondary responses is restored after the addition of third-party leucocytes to UV-irradiated MLCs[28], probably owing to APCs contained within the untreated leucocytes. Clustering between APCs and T cells involves interaction between MHC class II molecules and the T-cell receptor, between lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) and between CD2 and LFA-3, with additional stability provided by CD4 and CD8 (Fig. 2).

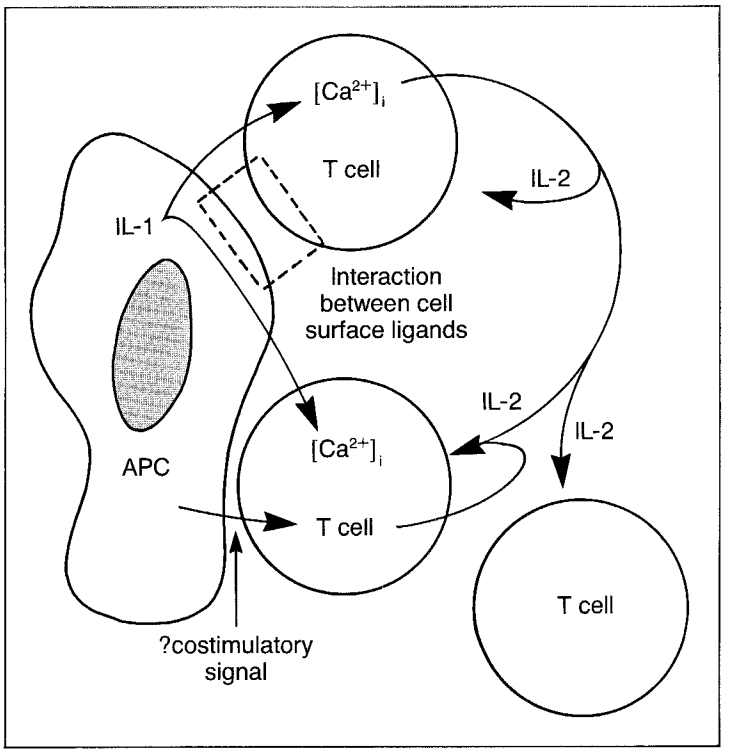

Fig. 1. *Induction of immune reactivity after mitogenic or allogeneic stimulation. Clustering of an APC with responding T cell results in IL-1 secretion and a rise in intracellular calcium ($[Ca^{2+}]_i$). Further intracellular signals induce transcription of the IL-2 gene. IL-2 secretion results in T-cell autostimulation and recruitment. This can be measured by increased [^{3}H]thymidine incorporation. The nature of a possible costimulatory signal, thought to be lost after UV irradiation or fixation in paraformaldehyde, is unknown. The dashed box refers to Fig. 2 and ligation of cell surface structure.*

UV irradiation could prevent normal cellular activation by interfering with the normal expression or function of cell surface ligands[15].

Studies on the expression of cell surface molecules have been performed in a number of species over a range of doses of UV radiation. In humans HLA-DR expression (but not A, B or C) declines after UV irradiation and disappears by 18 h after irradiation with 0.1 J m^{-2} of UVB[29]. At the opposite end of the spectrum, expression

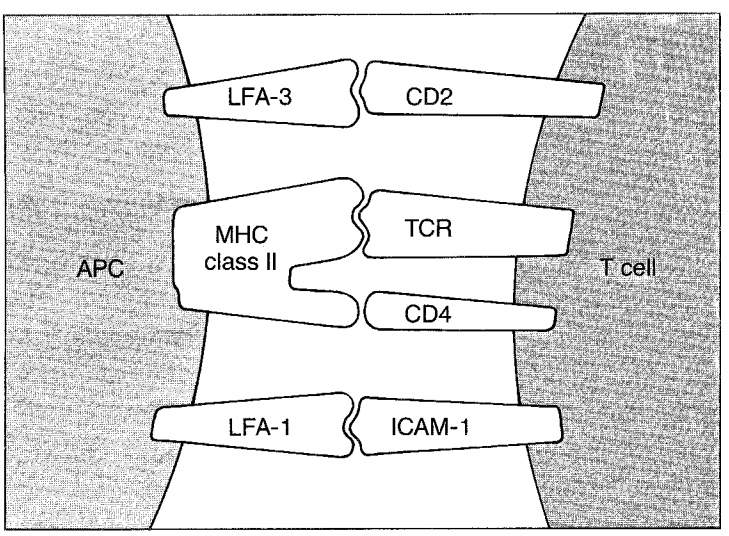

Fig. 2. *Schematic representation of the ligation of cell surface structures involved in APC–T-cell interactions.*

of HLA-DR, DQ and LFA-1 (CD11a, CD18) was found to be unchanged after 48 h when human peripheral blood mononuclear cells (PBMCs) were exposed to 3000 J m^{-2} of UVB[30]. Whether UVR caused qualitative changes in these and other cell surface structures is not known. On mouse spleen cells there is an initial loss of H-2K, I-A and I-E antigens after UV irradiation, which is accompanied by inhibition of I-A antigen synthesis. Thus the effect may be due, in part, to reduced re-expression of cell membrane H_2 molecules[31]. Expression of I-A antigens on cytoplasmic processes of DCs decreases to 30–40% of control values after culture for 48 h following UVC irradiation[32]; the expression of LFA-1 antigen on canine lymph node cells falls from 95% to less than 5% after UVB or UVC treatment[33].

Antigen presentation. APCs from the peritoneal exudate of UVB (1440 J m^{-2})-treated trinitrophenol-primed mice cannot induce hapten-specific delayed hypersensitivity responses in similarly irradiated syngeneic animals. This impairment of APC function is not observed when cells are obtained from nonirradiated donors[34]. Similarly, the proliferation of immune syngeneic T cells to C3H/He mouse epidermal cells pulsed with DNP_6–OVA, a dinitrophenolated derivative of ovalbumin, is reduced in a dose-dependent manner when the epidermal cells are exposed to UVB (50–200 J m^{-2})[35]. Proliferative responses were partially restored by addition of semi-purified epidermal-cell-derived thymocyte activating factor, which is physicochemically identical to IL-1 (Refs 35,36). Correction was also seen after addition of purified IL-1 or IL-2 (Ref. 37).

Calcium fluxes and lymphocyte activation. UVC-treated cells stimulated with phytohemagglutinin (PHA) or anti-CD3 monoclonal antibody fail to mobilize intracellular calcium when compared with controls[38]. The rise in intracellular calcium observed in normal lymphocytes after stimulation is accompanied by secretion of IL-1 and IL-2, which results in proliferation of T cells expressing an increased density of MHC class II molecules and IL-2 receptors. These changes are strongly inhibited after treatment of mononuclear cell suspensions with UVB (3000 J m^{-2})[33].

Despite extensive investigation, the precise nature of the 'UV-effect' is still unclear. UV-treated APCs may induce a state of operational tolerance in syngeneic T cells, although these cells may still proliferate under certain conditions. It has been suggested that this tolerance may result from loss of a membrane-bound, short-range costimulator activity, the nature of which is presently unknown[39].

In vivo *experimental immune suppression after UV irradiation*

Suppressor cells and factor(s). Tolerance to alloantigen and impaired contact hypersensitivity follows UV irradiation of the shaved dorsal skin of mice. It is interesting that this can occur when the skin sensitization site is remote from the irradiation site and penetration is minimal[1]. This could be explained by suppressor T (T_S) cells induced as a result of aberrant cutaneous antigen presentation[1].

When mice receive UV irradiation (10 000 J m^{-2} UVB) to shaved dorsal skin, the subsequent reduction in contact hypersensitivity is most marked 4 days later. Cell transfer to naive mice also reduces contact hypersensitivity. This phenomenon appears to be mediated by T_S cells of the Lyt-1^+2^- or Lyt-1^-2^+ phenotype[40]. Suppressor factor (SF) is released by freeze–thaw disruption of Lyt-1^+2^- T_S cells obtained from UVB-irradiated mice and these supernatants can impair contact hypersensitivity in naive animals[41]. SF is not related to IL-1 (Ref. 42).

T_S cells are not necessarily required for inhibitory activity. Oluwole *et al.*[43] have described permanent cardiac allograft survival in rats given UV-irradiated-DST, combined with peritransplant cyclosporin A. Serum from such recipients (presumably containing SF) prolonged allograft survival in naive syngeneic recipients[43].

Epidermal cells have also been shown to produce SF with a molecular mass of 15–50 kDa after UV irradiation[44] and this is consistent with the systemic suppression observed in UV-treated animals. Other factors are also involved since T_S cells are found in the spleen[40] and also produce SF[41].

Cytokines. UV irradiation has marked effects on the production of cytokines from mouse epidermal cells[45] and lymphocytes[46]: IL-2 and gamma-interferon (IFN-γ) are downregulated while IL-4 is upregulated by an IL-1β-dependent mechanism[46]. In addition, the secretion of IL-3, IL-6, tumour necrosis factor (TNF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) is induced by UV irradiation[45]. Despite these observations, SF activity has not yet been shown to result from the activity of any known cytokines.

Conclusion

UV irradiation has profound immunomodulatory effects: alloreactive and mitogen-induced responses are abolished; T_S cells and SF are produced and the secretion of cytokines is affected. Inactivation of DCs appears to be central to the loss of allostimulation. The use of UV irradiation may allow blood banks to issue non-immunogenic platelet transfusions and to facilitate clinical transplantation across histocompatibility barriers without graft rejection and the use of immunosuppressive agents. Not only could clinical GVHD be abolished, but it might also become possible to transplant between mismatched individuals, thereby expanding the pool of readily available donors. UVB is biologically most relevant, since it spares the function of specialized cells; whereas UVC damages haemopoietic stem cells.

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