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P-glycoprotein and alloimmune T-cell activation

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Abstract

P-glycoprotein (P-gp), the human multidrug resistant (MDR1) gene product and cancer multidrug resistance-associated adenosine triphosphate (ATP)-binding cassette (ABC) transporter, is physiologically expressed on peripheral blood mononuclear cells, but its role in cellular immunity is only beginning to be elucidated. A role of P-gp in the secretion of several T-cell and antigen presenting cell-derived cytokines has been described, and additional functions of the molecule have been identified in lymphocyte survival and antigen presenting cell differentiation. Taken together, these findings provide compelling evidence that P-gp serves several distinct functions in the initiation of primary immune responses, and a critical role of the molecule in functional alloimmune responses is now established. Here, we will review the current understanding of P-gp function in alloimmune T-cell activation via both T-cell and antigen presenting cell-dependent mechanisms, which is relevant to the field of clinical transplantation, where P-gp has been found to be a marker of acute and chronic allograft rejection. Indeed, current in vitro findings raise the possibility that P-gp could represent a novel therapeutic target in acute and chronic allograft rejection, the major causes of allograft dysfunction and ultimate graft loss.

Keywords

P-glycoprotein; T-cell activation; Antigen presentation

1. Introduction

P-glycoprotein (P-gp) is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of active transporters, which includes, among others, the multidrug resistance related protein (MRP), the cystic fibrosis transmembrane conductance regulator (CFTR) and the transporter of antigen processing (TAP) [1]. P-gp is encoded by a multigene family in higher eukaryotes. In humans, P-gp is encoded by two linked genes on chromosome 7q21.1, multidrug resistant (MDR1) and MDR3, and in rodents by three linked genes. Among those, the duplicate *mdr1a* and *mdr1b* murine genes encode P-gps, which have been described as structurally and functionally homologous to the human MDR1 gene product [2].

Overexpression of MDR1 P-gp has been associated with the MDR phenotype of certain mammalian solid tumors and hematological malignancies [3]. MDR1 P-gp functions as an ATP-dependent drug efflux pump in such tumors, conferring cellular resistance to cytotoxic

xenobiotics by reducing intracellular drug accumulation and, as a consequence, drug toxicity. P-gp drug efflux function can be inhibited by a large variety of chemical compounds [4,5], including small molecule modulators such as the calcium channel blocker verapamil, the triparanol analog tamoxifen and cyclic peptides such as the immunosuppressive agent cyclosporine A and its non-calcineurin inhibitory analog PSC833 [6,7]. In addition, P-gp function can be modulated by P-gp-specific monoclonal antibodies (mAbs) [8] and antisense oligonucleotides directed at the P-gp encoding gene [9,10].

Based on this role in cancer multidrug resistance, MDR1 P-gp has been the subject of intensive investigation in the field of oncology. In comparison, relatively little is known about the physiological function(s) of the P-gp family members. In humans, MDR3 P-gp functions as a lipid translocase, and specific MDR3 gene mutations have been identified in familial intrahepatic cholestasis [11]. MDR1 P-gp, in addition to being overexpressed in cancer cells, is widely expressed in normal, predominantly secretory human tissues [12] and in cells of the immune system, and has been implicated in the secretion and transmembrane transport of various molecules, including xenobiotics, peptide molecules, steroid compounds, phospholipids [13] and, as we will discuss, a subset of cytokines. There is now accumulating evidence that MDR1 P-gp and related molecules, by mediating or regulating basic and diverse cellular functions, may serve critical physiologic roles in cellular differentiation, proliferation, survival and immunity [14].

Among human bone marrow-derived cells, P-gp has been found to be expressed in hematopoietic CD34⁺ stem cells, thymocytes, natural killer (NK) cells, lymphocytes and antigen presenting cells (APCs) [15–20] and specific roles of the molecule in human cellular immunity including alloimmune responses, inferred from in vitro studies utilizing both pharmacological or P-gp-specific functional inhibitors, are now well recognized. In alloimmune responses following organ transplantation, the primary initial event that ultimately leads to graft rejection is allorecognition, that is T-cell recognition of alloantigen, in particular antigens of the major histocompatibility complex (MHC). It has been demonstrated that CD4⁺ T-cells are essential for initiating allograft rejection [21]. Two distinct, yet not mutually exclusive pathways of allorecognition by CD4⁺ T-cells have been described: The *direct* pathway, in which T-cells recognize intact allo-MHC molecules on the surface of allogeneic donor APCs, and the *indirect* pathway, in which T-cells recognize processed allopeptide in a self-restricted manner presented by self-APCs [22]. Since it is now known that P-gp serves critical roles in both T-cells and APCs in vitro, it is quite likely that the molecule may function also in direct and indirect allorecognition in vivo, and thus may be relevant to the processes of both acute and chronic allograft rejection. This has provided the rationale for further elucidation of the function of P-glycoprotein in cellular immunity in general, and alloimmunity specifically. Here, we will present a review of the current understanding of P-gp expression and function in human T-cells and APCs and of the role of the molecule in the interactions among these immune cells, which are critical to the alloimmune response in the process of allograft rejection.

2. P-gp expression in resting and activated T-cells

Multiple assays have been developed and employed for the detection and functional analysis of the MDR1 gene and its corresponding gene product P-gp [23]. Gene-specific methods directed at detection of MDR1 messenger ribonucleic acid (mRNA), including the polymerase chain reaction (PCR), Northern analysis and RNase protection assays have been used to study P-gp expression; however, P-gp expression and function has most commonly been examined at the protein level using Western analysis, immunohistochemistry, fluorescent dye transport studies, most commonly involving rhodamine-123 (Rh-123) [15,24] or immunostaining-based flow cytometry. These assays differ in their specificity for

MDR1 P-gp, and it is now well-recognized that certain fluorescent dye probes are transport substrates for related ABC transporters in addition to MDR1 P-gp. Furthermore, among the panel of well-characterized anti-P-gp mAbs that recognize either internal (C-219, C494, JSB-1) or external (Hyb-612, Hyb-241, MRK16, MRK17, 265/F4, 4E3.16, UIC2) epitopes of the transporter [23,25–29], only a subset is thought to be highly specific for MDR1 P-gp, including Hyb-241 and MRK-16, whereas others, such as C219, are known to bind epitopes conserved among all known P-gp molecules and additional ABC transporters. These limitations of the currently available methods for the specific study of the MDR1 gene and its gene product have to be recognized when critically examining the evidence for P-gp expression and function in immune cells, and account in part for the considerable controversy surrounding these issues. Table 1 lists the most commonly used anti-P-gp mAbs and summarizes the respective findings with regard to detection of P-gp expression in human immune cells and functional effects of mAb-mediated P-gp blockade.

With regard to resting T-cells, Neyfakh et al. [15,24] initially demonstrated that normal human and murine peripheral lymphocytes efflux fluorescent dyes and Chaudhary and Roninson [16] later confirmed these findings. Staining of normal human bone marrow cells with Rh-123 and subsequent incubation in Rh-123-free medium in the absence or presence of the pharmacological P-gp inhibitors verapamil, reserpine or PAK-104P revealed that in the absence of inhibitors, 60–65% of lymphoid cells and 20–30% of blast cells had effluxed Rh-123 (Rh-123^{dull}), but almost no Rh-123^{dull} cells were detected among the granuloid population. In the presence of P-gp inhibitors, however, Rh-123 efflux was inhibited and all of the bone marrow cells were Rh-123^{bright}. These investigators then labeled Rh-123-stained cells with the anti-Pgp mAbs MRK16 or UIC2 and found P-gp to be expressed on approximately 55 to 65% of lymphoid bone marrow cells and that this expression correlated inversely with dye retention in lymphoid cells. These studies revealed further that it was the CD34⁺ subset of lymphoid cells, which had increased expression of P-gp along with low dye accumulation, that is increased dye efflux. Based on the additional finding that these CD34⁺ cells were HLA-DR^{low} and CD33[−], two known features of stem cells, it was suggested that P-gp expression was associated with the stem cell phenotype. In a subsequent study, dye efflux was assessed in a population of peripheral blood lymphocytes [17]. In this investigation, efflux of Rh-123 and DiOC2 from human peripheral blood lymphocytes was used in conjunction with either P-gp inhibitors or the anti-P-gp mAb UIC2 to analyze the correlation between dye efflux and P-gp expression. Peripheral blood mononuclear cells (PBMC) were loaded with dye at 4°C and then incubated in dye-free media at 37°C for 3 hours. When incubated alone, 50 to 70% of cells had effluxed the dye. In contrast, incubation at 4°C to prevent ATP hydrolysis or in the presence of P-gp inhibitors resulted in nearly all the cells being replete with dye by flow cytometry, suggesting decreased dye efflux, and 40 to 65% of PBMC were shown to express P-gp and the P-gp-specific mAb UIC2 inhibited dye efflux, suggesting that it was P-gp-mediated. These investigators then assessed the patterns of P-gp expression in specific PBMC subsets and found that 80 to 90% of CD8⁺ cells effluxed dye and that a similar percentage (70 to 80%) of these cells stained positive with UIC2. Even greater percentages were found in the CD56⁺ natural killer cell population with 90 to 95% of cells effluxing dye and staining positive with UIC2. The CD4⁺ cell population, in contrast, had the least dye efflux, at 40 to 50%, and the lowest number of cells that stained positively for UIC2 (30 to 40%). CD14⁺ monocytes, finally, appeared to lack functionally active P-gp by these studies. Klimecki et al. [30] utilized another antibody, MRK-16, and found the highest levels of P-gp expression in CD56⁺ NK cells (72%) and CD8⁺ suppressor cells (48%), and lower expression in CD4⁺ T-cells (33%), CD19⁺ B-cell (30%) and CD14⁺ monocyte cells (19%). In addition, these investigators analyzed MDR1 mRNA expression, finding P-gp highly expressed in CD56⁺ and CD8⁺ cells, moderately in CD4⁺ and CD19⁺ cells, and at low levels in CD14⁺ cells. In studies from our laboratory [31], we utilized the MDR1 P-gp-specific mAb Hyb-241, considered most specific for MDR1 P-

gp among available anti-P-gp antibodies [32], and flow cytometry to assess P-gp expression by subsets of resting PBMCs and found surface P-gp expressed on 21% of CD3⁺ T-cells and 18% of purified CD4⁺ T-cells. In addition, Hyb-241 recognized surface P-gp on 84% of CD14⁺ APCs. Furthermore, dye efflux studies utilizing calcein-AM (calcein acetoxymethyl ester) in the presence of both pharmacological or specific P-gp inhibitors revealed that P-gp was functional in CD4⁺ T-cells and CD14⁺ APCs. While untreated or isotype control Ab-treated CD4⁺ cells effluxed 47% of dye at 90 minutes, tamoxifen- or Hyb-241-treated cultures exhibited a decreased dye efflux capacity of 15%, and a similar effect could be demonstrated in CD14⁺ APCs.

In aggregate, these findings by multiple investigators provide a consistent picture of expression of functionally active P-gp on bone marrow-derived lymphoid progenitor cells and, to lower degrees, on differentiated resting human T-cell subsets, including CD4⁺ and CD8⁺ cells. Furthermore, the demonstrated presence of MDR1 mRNA in CD14⁺ monocytes, the detection of surface P-gp expression on these cells by two distinct anti-P-gp mAbs and the observed dye efflux capacity of these cells establish that P-gp is functionally expressed in APCs.

Additional studies have been directed at assessing P-gp regulation in the course of T-cell activation. Gupta et al. [33] assessed the percentages of P-gp⁺ cells T-cell subsets with and without polyclonal phytohemagglutinin (PHA) stimulation. P-gp⁺ cells in these studies made up 5–10% of freshly isolated T-cells, specifically 3% of CD4⁺ cells and 10–20% of CD8⁺ T-cells. Upon PHA stimulation, 35–75% of CD3⁺, 16–40% of CD4⁺ and 54–100% of CD8⁺ cells expressed P-gp. Analysis of the kinetics of P-gp expression induced by PHA activation revealed a time-dependent augmentation in CD3⁺ T-cells, which peaked at 48 hours. Examination of the kinetics of MDR1 mRNA expression revealed low levels of MDR1 mRNA in unstimulated cells, which also increased in a time-dependent fashion, peaking at 24 hours. Mu et al. [34] also investigated the relationship between T-cell activation and P-gp expression. Flow cytometry using the anti-P-gp mAb 4E3 showed 0.7% of lymphocytes expressing P-gp among the resting T-cell population, augmented to 3.3% expression after 24 hours of PHA-mediated mitogenic stimulation. In our studies, PHA stimulation did not significantly augment CD4⁺ T-cellular P-gp expression at 24 hours, as assessed by flow cytometry using the Hyb-241 mAb. In addition, stimulation with allogeneic APCs did not significantly increase P-gp expression by CD4⁺ T-cells at 24 hours [31]. Together, these studies indicate that P-gp is expressed by both resting and activated T-cells, and that prolonged polyclonal stimulation >24 hours can significantly enhance P-gp surface expression on both CD4⁺ and CD8⁺ human T-cells. These findings have generated interest to elucidate the role of P-gp in normal immune function.

3. P-gp function in T-cells

P-gp function in human T-cells has been assessed with regards to possible roles of the molecule in cytokine secretion, T-cell survival and differentiation, and cytotoxic T-cell function, all of which are critical components of the alloimmune response. With regard to cytokine expression and secretion in human T-cells, an initial report by Drach et al. [35] indicated that the anti-P-gp mAb UIC2, along with the pharmacological P-gp inhibitors verapamil and tamoxifen, inhibited IL-2, IL-4 and IFN- γ production in culture supernatants of PHA-activated human PBMC. Treatment of PHA-activated lymphocytes with 1 μ g/ml verapamil reduced IL-2 levels by 5–10% compared to controls and 10 μ g/ml verapamil resulted in a reduction to 20–40% of controls. Similarly, tamoxifen reduced IL-2 levels by 2–10% at 1 μ M and 70–95% at 10 μ M concentrations. When activated lymphocytes were treated with UIC2, IL-2 levels were significantly reduced to 16% of controls. This inhibitory effect of UIC2, however, was found to be epitope-specific, since the MRK-16 mAb, which

recognizes a different P-gp epitope, did not significantly suppress cytokine secretion. IL-2 mRNA expression was assessed in PHA-stimulated lymphocytes both in the presence and absence of P-gp inhibitors and was found to be equivalent in the two groups, suggesting that the mechanism of inhibition was not at the transcriptional level. This was in contrast to a significant decrease in IL-2 mRNA expression seen in lymphocytes stimulated in the presence of cyclosporine A, a known inhibitor of IL-2 gene transcription. The P-gp inhibitors blocked, however, IL-2 release, suggesting that P-gp functioned at the post-transcriptional level of cytokine transport and secretion. Raghu et al. [36] also studied the effects of the mAb UIC2 on cytokine expression in PHA-stimulated PBMC and found a significant two-fold reduction in IL-2 levels with treatment with 5 µg/ml UIC2 and a greater than three-fold reduction at 100 µg/ml concentrations of Ab. While MRK-16 also resulted in suppression of IL-2 secretion into the culture supernatants, UIC2 exhibited a greater inhibitory effect. To address the question of whether IL-2 suppression was due to P-gp blockade-mediated inhibition of PBMC activation or was in fact a result of P-gp transport inhibition, various markers of T-cell activation were assessed, including calcium flux and the expression of CD69 at 6 hours. Neither of these activation markers was inhibited, suggesting that cytokine suppression was a result of P-gp blockade-mediated efflux inhibition. In agreement with the findings of other groups, there was no decrease in IL-2 mRNA expression in PHA-stimulated PBMC despite treatment with anti-P-gp mAb, also suggesting that the effect was due to suppression of P-gp-mediated IL-2 transport, rather than due to changes at the transcriptional level. Gollapudi et al. [37] also found that the anti-P-gp mAb UIC-2 inhibited IL-2 secretion by anti-CD3-stimulated PBL in a concentration-dependent manner; however, these authors also reported that human Jurkat T-cells lacking P-gp and MDR1 gene-transfected Jurkat T-cells produced comparable amounts of IL-2 into culture supernatants upon anti-CD3 polyclonal stimulation. Furthermore, anti-CD3-activated P-gp⁺ or P-gp⁻ CD4⁺ and CD8⁺ T-cells, separated on the basis of MRK16 expression, produced similar amounts of IL-2 upon polyclonal activation. Thus, it was concluded that P-gp was not required for IL-2 secretion by human T-cells and that anti-P-gp-induced inhibition of IL-2 secretion might be due either to binding of the UIC-2 mAb to additional molecules other than P-gp and/or to possible cell loss via anti-P-gp antibody-induced apoptosis of activated T-cells. While cross-reactivity of the anti-P-gp mAb UIC-2 or MRK16 with another molecule has not been demonstrated to date, subsequent studies by these investigators confirmed that UIC2 indeed induced apoptosis and inhibited lymphocyte proliferation in CD3-activated human lymphocytes. Exogenous IL-2 supplementation did not alter this effect, and induction of apoptosis was achieved with concentrations of UIC2, which had no significant effects on IL-2 production, suggesting that anti-P-gp-induced apoptosis was not due to IL-2 deprivation. These findings of an anti-apoptotic role of P-gp in human T-cells parallel such a role of the molecule in other cell types, where P-gp has been described to confer resistance to caspase-dependent but not caspase-independent apoptosis [38–40], reversible by anti-P-gp mAbs or the P-gp inhibitor verapamil. Thus, recent investigations have challenged earlier notions that P-gp functions as an IL-2 transporter in human T-cells, but instead indicate that P-gp serves a regulatory role in T-cell survival, and hence possibly in the selection of an appropriate lymphocytic repertoire in the course of immune activation. Altered cytokine expression profiles, such as have been observed under conditions of P-gp blockade by many investigators, may in this regard reflect phenotypic differences in the selected T-cell repertoire, rather than resulting from inhibition of P-gp-mediated transport. Our laboratory has also assessed the effects of pharmacological and specific P-gp inhibition on human T-cellular cytokine production; however, unlike the aforementioned studies, which have addressed the role of P-gp in cytokine secretion as a result of polyclonal activation, we have examined the role of P-gp in cytokine production as a result of alloimmune stimulation [31]. In our studies, we found that the pharmacological P-gp inhibitor tamoxifen and the MDR1 P-gp-specific mAb Hyb-241 blocked alloantigen-dependent IL-2, IFN-γ and TNF-α production and T-cell proliferation in

the mixed lymphocyte reaction (MLR). In contrast, Hyb-241-mediated MDR1 P-gp-specific blockade had no significant effect on mitogen-induced T-cell proliferation. MLR-induced T-cell proliferation could be restored in P-gp inhibitor-treated cultures by the addition of exogenous IL-2, pointing to a critical role of P-gp in alloimmune T-cell activation prior to IL-2 secretion. Selective blockade of P-gp in purified CD4⁺ T-cell populations prior to MLR co-culture also resulted in inhibition of IFN- γ and tumor necrosis factor (TNF)- α production, demonstrating a direct involvement of T-cellular P-gp in alloimmune activation. In contrast to the findings by Gollapudi et al. [37] of an induction of apoptosis by P-gp inhibitors in CD3-stimulated T-cells, we did not detect increased rates of apoptosis above control among alloimmune-stimulated T-cells in the presence of P-gp inhibitors. If P-gp inhibition promoted activation-induced cell death preferentially during early stages of T-cell activation, however, when the clonal size of alloreactive, IL-2-producing T-cells among the T-cell population as a whole is relatively small, it would be conceivable that increased rates of apoptosis in this subset might not be easily detected. Another mechanism, however, by which CD4⁺ T-cellular P-gp may regulate T-cell activation and IL-2 production, relates to a function of the molecule in IFN- γ -dependent mechanisms of Th1 (T-helper cell-1) cell differentiation suggested by our findings. In contrast to the emerging evidence that P-gp is not required for IL-2 transport, the presumptive role of the molecule in IFN- γ secretion by human T-cells has not been challenged. In our studies, we have found that specific P-gp blockade of CD4⁺ T-cells alone resulted in diminished IFN- γ secretion by CD4⁺ alloreactive T-cells, consistent with the previously postulated role of P-gp in the cellular transport of this cytokine. In addition, selective P-gp inhibition on CD4⁺ T-cells diminished IL-12 production by allogeneic APCs, suggesting that T-cellular P-gp blockade dysregulated the IFN- γ /IL-12 positive feedback loop and, as a result, T-cell differentiation into IL-2/TNF- α -producing Th1 cells. Taken together, the studies of P-gp function in human T-cells have thus identified several critical roles of the molecule in T-cell activation, including anti-apoptotic functions in the course of polyclonal T-cell activation and cytokine (IFN- γ)-dependent mechanisms of Th1 differentiation in the course of alloimmune T-cell activation. The finding of a role for P-gp in human Th1 differentiation is paralleled by findings in murine T-cells where P-gp expression within the CD4⁺ T-cell subset correlated with differential cytokine expression [41]. These investigators characterized a Rh-123-effluxing subset of preactivated CD4⁺ T-cells that would have been considered naïve on the basis of its surface phenotype (CD45RB⁺, CD44^{low}, CD62L⁺, CD69⁻, CD25⁻), which secreted higher levels of the Th1 cytokines IL-2 and IFN- γ and had a higher rate of proliferation in response to various activation stimuli, including concavalin A (ConA), anti-CD3 mAb and allogeneic cells, than their P-gp⁻ counterparts, but were unable to mount a similar IL-4 or IL-10 response to various polyclonal stimuli. These findings in wild-type mice contrast, however, those obtained from studies of immune function in *mdr1a* and *mdr1b* knockout (KO) or *mdr1a/mdr1b* double KO mice, indicating a possible redundancy of murine P-gp function and thus the possibility that murine *mdr1a/1b* function may differ from MDR1 P-gp function in humans.

Cytokine gene expression and cytokine levels in *mdr1a* and *mdr1b* KO mice has been assessed by Gollapudi et al. [37]. In these studies, splenocytes from wild type, *mdr1a* KO and *mdr1b* KO mice were stimulated with anti-CD3 mAb for 24 and 48 hours and culture supernatants were analyzed for IL-2, IFN- γ , IL-4 and IL-10 levels by enzyme-linked immunosorbent assay (ELISA) and cellular RNA extraction. No significant differences in IL-2 mRNA levels were found in lymphocytes from the three groups, and the levels of the IL-2, IFN- γ , IL-4 and IL-10 cytokines were similar in both groups of KO and wild type mice, suggesting that P-gp in mice was not required for cytokine secretion. In addition, proliferative responses to stimulation with anti-CD3 mAb or ConA were found to be similar in wild type and *mdr1a* or *mdr1b* KO mice. Eisenbraun and Miller [42] obtained similar results when comparing T-cell responses in wild type mice with those in *mdr1a* KO mice,

with no differences noted with regard to proliferation or IL-2, IL-4, IL-5, IL-10 and IFN- γ secretion in response to polyclonal stimuli. Furthermore, T-cells from *mdr1a* KO mice generated similar allospecific cytotoxic responses as T-cells derived from wild type mice, in contrast to findings in human T-cells, where a role for P-gp in cytotoxic T-cell function has been established [33]. Thus, *mdr1a/1b*-deficient mice appear limited as a model system from which inferences can be made about MDR1 P-gp function in human T-cells.

4. P-gp expression and function in APCs

Alloimmune T-cell activation results from interactions of alloreactive T-cells with either donor APCs (direct pathway of allorecognition) or self-APCs (indirect pathway). Thus, P-gp may function in alloimmune T-cell activation not only via its roles on the T-cell, but also via APC-dependent mechanisms. Several investigators, including our laboratory, have reported P-gp expression by CD14⁺ monocytes [17,19,30,31,43], and P-gp functions on these cells in ATP-dependent transport phenomena [31]. There exists considerable controversy regarding the precise degree of P-gp expression in human APCs as outlined above, and the reported differences in the expression pattern may relate to the differential P-gp binding affinities of the various mAbs used, which are directed to different P-gp epitopes. The detection of major proportions of P-gp-expressing APCs by two of the most specific anti-P-gp mAbs MRK 16 (19% positivity) [30] and Hyb-241 (84% positivity) [31] and, furthermore, the detection of MDR1 mRNA in human APCs [30], demonstrate, however, that MDR1 P-gp is significantly expressed among APCs. The physiologic function(s) of APC-associated P-gp are, however, incompletely understood. Randolph et al. [44] have reported a role of monocyte-associated P-gp in the differentiation of these cells into myeloid-derived dendritic cells, a function that was blocked by anti-P-gp mAbs and pharmacological P-gp inhibitors. In our laboratory, we have specifically addressed the role of APC-expressed P-gp in alloimmune T-cell activation and cytokine secretion. Preincubation of irradiated stimulator APCs with the anti-P-gp mAb Hyb-241 before MLR co-culture (selective blockade of APC-expressed P-gp) significantly inhibited allogeneic CD4⁺ T-cell proliferation and markedly inhibited IL-12 and TNF- α secretion into MLR culture supernatants. To further examine the effects of P-gp blockade on IL-12 and TNF- α secretion, we analyzed the effects of specific P-gp inhibition on intracellular TNF- α and IL-12 accumulation in purified CD14⁺ cell populations. Hyb-241-mediated P-gp blockade did not induce detectable intracellular TNF- α levels and secretion of TNF- α by lipopolysaccharide (LPS)-stimulated monocytes was unaltered. In contrast, P-gp inhibition augmented intracellular IL-12 accumulation in LPS-stimulated monocytes, while significantly reducing IL-12 concentrations in supernatants of treated cultures, suggesting a novel role for P-gp in IL-12 transport. These results demonstrated that P-gp functions in alloimmune T-cell activation not only via CD4⁺ T-cell-dependent, but also via APC-dependent pathways, as IL-12 is critical for T-cellular IFN- γ production and Th1 differentiation.

5. Is P-gp needed in human alloimmune responses?

Current evidence thus indicates several distinct functions of MDR1 P-gp in the human alloimmune response and suggests critical roles of the molecule in human CD4⁺ T-cell activation via either the direct or the indirect pathways of allorecognition. Furthermore, a role of P-gp in CD8⁺ T-cell-mediated cytotoxic functions has been established. That P-gp may also be a critical component of human alloimmune responses *in vivo* is suggested by clinical observations, which have correlated with P-gp overexpression by graft-infiltrating or circulating immune cells with both frequency and severity of allograft rejection in human transplant recipients [45,46]. Furthermore, there is emerging evidence that well-characterized pharmacological P-gp inhibitors, such as tamoxifen, are immunomodulatory in

vivo. Thus, P-gp may represent a novel therapeutic target in acute and chronic allograft rejection, and further studies regarding the role of P-gp in these processes are warranted.

Abbreviations

ABC	ATP-binding cassette
APC	antigen presenting cell
ATP	adenosine triphosphate
CFTR	cystic fibrosis transmembrane conductance regulator
ConA	concanavalin A
ELISA	enzyme-linked immunosorbent assay
KO	knockout
LPS	lipopolysaccharide
mAB	monoclonal antibodies
MDR	multidrug resistant
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
MLR	mixed lymphocyte reaction
MRP	multidrug resistance related protein
NK	natural killer
PBMC	Peripheral blood mononuclear cells
PCR	polymerase chain reaction
P-gp	P-glycoprotein
PHA	polyclonal phytohemagglutinin
Rh-123	rhodamine-123
TAP	transporter of antigen processing
Th1, TNF	tumor necrosis factor
Th1	T-helper cell-1
calcein-AM	calcein-acetoxymethyl ester

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Table 1

Immune cell binding and modulation by Anti-P-gp antibodies

Anti-P-gp antibody	Epitope site	Reported function	References
MRK16	External	Blocks migration of mononuclear phagocytes	[44]
		Staining CD56 ⁺ > CD8 ⁺ , CD4 ⁺ and CD19 ⁺ cells > CD14 ⁺ cells. No staining of CD15 ⁺ cells	[30]
		Staining of 55–65% of the lymphoid bone marrow cells, correlated with dye retention	[16]
		Expression on CD8 ⁺ > CD4 ⁺ cells. Inhibits T cell cytotoxic function	[33]
		Blockade induces decreased IL-2 levels, but not IL-2 mRNA	[36]
		Inhibits NK cell-mediated cytotoxicity	[47]
UIC2	External	Blocks migration of mononuclear phagocytes	[44]
		Staining of 55–65% of the lymphoid bone marrow cells; correlation with dye retention	[16]
		Inhibits IL-2, IL-4, IFN- γ not IL-6	[35]
Hyb241	External	Decreases IL-2 levels but not IL-2 mRNA	[36]
		Inhibits alloimmune IL-2, IFN- γ and TNF- α and IL-12 production via both APC and CD4 ⁺ dependent pathways	[31]
4E3	External	In CD8 ⁺ cells, 4E3 is more specific for P-gp than MRK16	[34]
		Blocks transendothelial migration of mononuclear phagocytes	[44]
C219	Internal	Staining of CD4 ⁺ , CD8 ⁺ , CD14 ⁺ , CD19 ⁺ , CD56 ⁺ cells, not granulocytes	[19]