Immunology 1997 **90** 57–65

Desialylation of T lymphocytes overcomes the monocyte dependency of pokeweed mitogen-induced T-cell activation

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SUMMARY

Activation of T lymphocytes by pokeweed mitogen (PWM) is strictly monocyte (Mo)-dependent and results in T-cell mitogenesis and interleukin-2 (IL-2) secretion, coupled with an inability to utilize IL-2 due to an impaired expression of functional IL-2 receptor (IL-2R). Such IL-2R impairment could arise in PWM-activated T cells themselves or, alternatively, be the result of Mo-derived influences, as it is known that PWM binds Mo strongly and does not or poorly binds lymphocytes, and Mo becomes rapidly destroyed in PWM-stimulated cultures of blood mononuclear cells or T cells plus Mo. The present study investigated these possibilities. The results show for the first time that desialylation of T lymphocytes strongly increases their PWM-binding capacity and, in addition, overcomes the Mo requirement for PWM to induce T-cell mitogenesis and IL-2 secretion. Such secreted IL-2 levels were even higher that those found in cultures of Mo-dependent PWM-activated T lymphocytes but, similarly to the latter, PWM-activated desialylated purified T lymphocytes exhibited negligible high-affinity IL-2 binding capacity and an inability to utilize the IL-2 they produced. These effects were not due to desialylation itself, as indicated by data obtained with peanut agglutinin, a lectin that becomes strongly reactive with desialylated T lymphocytes. The data clearly indicate the existence of PWM-related events capable of impairing the expression of functional IL-2R without affecting IL-2 secretion, and indicate that such events are due to mechanisms arising at the level of PWM-activated T cells themselves.

INTRODUCTION

The lectins phytohaemagglutinin (PHA) and concanavalin A (Con A) and CD3 monoclonal antibody (mAb) are utilized extensively as *in vitro* inducers of monocyte (Mo)-dependent interleukin-2 (IL-2)-mediated T-cell proliferation.[1–3] In contrast, pokeweed mitogen (PWM) is widely used for *in vitro* induction of Mo- and T-cell dependent polyclonal differentiation of B cells. In such PWM-driven B-cell differentiation, both cognate $CD4^+$ T-cell–B-cell interactions and PWM-induced IL-2 and IL-6 play a critical role.[4–8] Although it is also well known that PWM induces Mo-dependent T-cell mitogenesis,[9–12] the analysis of PWM-induced T-cell activation has received little attention. Nevertheless, there is evidence[11,12] that such activation should be considered separately from the current model of T-cell activation based on the use of PHA, Con A and CD3 mAb[1,2] because: (1) the Mo dependency of PWM-induced T-cell mitogenesis is not overcome by the presence of exogenous cytokines (IL-2, IL-1, IL-1 + IL-2 or IL-4), even using immobilized PWM,[12] while this is the case for Con A, PHA and CD3 mAb;[1,2] (2) T cells activated with PWM in the presence of Mo secrete high levels of IL-2 but undergo IL-2-independent DNA synthesis[11,12] as they are unable to utilize the IL-2 they produce due to a negligible expression of high-affinity (HA) IL-2R.[12] As we have already discussed,[12] this pattern of high IL-2 production coupled with IL-2 non-utilization by Mo-dependent PWM-activated T cells is likely to account for the capacity of PWM to promote T-cell-dependent B-cell differentiation, a property that is not shared by the classic T-cell lectins PHA and Con A.

The impaired expression of HA IL-2R on PWM-activated T cells is not coupled with a reduced gene transcription of IL-2R α (CD25) or IL-2R β (CD122, p75) chains, suggesting the existence of events inhibiting IL-2R subunit expression post-transcriptionally.[12] Such events could originate at the level of PWM-activated T cells themselves or, alternatively, be the consequence of Mo-derived effects because in PWM-stimulated cultures of peripheral blood monocuclear cells (PBMC) or $CD4^+$ T cells plus Mo, Mo are rapidly

Received 23 April 1996; revised 18 August 1996; accepted 18 September 1996.

Abbreviations: BDMC, B-cell-depleted mononuclear cells; FITC, fluorescein isothiocyanate; HA, high-affinity; LGL, large granular lymphocytes; mAb, monoclonal antibody; Mo, monocytes; Nase, neuraminidase; PBMC, peripheral blood mononuclear cells; PBT, purified blood T lymphocytes; PE, phycoerythrin; PMA, phorbol myristate acetate; PNA, peanut agglutinin; PWM, pokeweed mitogen; SA, streptavidin.

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destroyed,[10,13] a phenomenon that appears to be caused by activated $CD4^+$ T cells.[13] This destruction of Mo by activated T cells may provide negative influences (prostaglandins, oxygen or nitrogen intermediates, lysosomal enzymes) that, in turn, could act on activated T cells by impairing their expression of functional IL-2R, a plausible possibility as prostaglandins inhibit IL-2Rα chain expression post-transcriptionally.[14] The aim of the present study was to gain insight about these possibilities. We first analysed the binding of PWM to T lymphocytes, as this information is lacking and is important for that purpose. It should be noted that while the analysis of T-cell molecules interacting with PHA and Con A has been the subject of a number of studies,[15] the analysis of T-cell molecules reacting with PWM not only is lacking, but it has been reported that PWM mainly binds Mo, while it does not or poorly binds T lymphocytes,[9,10] and it has been suggested that a PWM–Mo interaction could be strictly required for PWM to bind and activate T cells.[10]

MATERIALS AND METHODS

Lectins, antibodies, cytokines and reagents

PWM, Con A and peanut agglutinin (PNA), either unconjugated or fluorescein isothiocyanate (FITC)-, biotin- or agarose-conjugated, as well as FITC-conjugated PHA were obtained from Sigma Chemicals (Taufkirchen, Germany). Bovine serum albumin (BSA) and PWM were coupled to CNBr-activated Sepharose 4B (Pharmacia, Barcelona, Spain), as reported elsewhere.[12] PHA for cell cultures was from Welcome Diagnostics (Dartford, UK). Anti-PWM rabbit IgG, unconjugated or biotin-conjugated,[12] FITC-conjugated anti-rabbit IgG swine antibodies (Kakopatts, Barcelona, Spain), FITC-conjugated anti-mouse immunoglobulin goat antibodies (Sigma), streptavidin (SA)–FITC (Tago Inc., Burlingame, CA) and SA–phycoerythrin (PE) (Becton Dickinson España SA, Madrid, Spain) were used for cell surface staining. The following mAb, either produced in our laboratory[12,16,17] or donated by other investigators, were used for cell complement-mediated lysis of cells and/or for cell surface staining (either biotin-labelled or uncongugated): CD20 (B-C1), CD24 (B-C2), CD14 (Cris-6), CD3 (Cris-7), CD4 (Edu-2), CD8 (109–2D4), EDU-1 mAb, EDU-1 (anti-HLA-class II molecules); the CD122 mAb, TU27,[18] (anti-IL-2R β chain) donated by Dr K. Sugamura (Tohoku, Japan); the CD25 mAb, MAR108,[19] donated by Dr M. López-Botet (Madrid, Spain); the CD98 mAb, FG1/8,[12,17] donated by Dr F. Sanchez-Madrid (Madrid, Spain). Commercially available CD5 (Leu-1), CD3 (Leu-4), CD56 (Leu-19), CD19 (Leu-12) and CD25 (IL-2R) mAb (Becton Dickinson), either unconjugated or FITC- or PE-conjugated, were also used for cell surface staining. Phorbol myristate acetate (PMA) and recombinant IL-2 were, respectively, from Sigma and Boehringer Manheim GmbH (Manheim, Germany).

Cells

PBMC were separated (Ficoll gradient, density 1077) from fresh heparinized venous blood of adult healthy volunteers. To obtain purified blood T lymphocytes (PBT), PBMC were subjected to complement-mediated lysis[12,16,17] using mAb B-C1 (CD20), B-C2 (CD24), CD14 (Cris-6) and EDU-1 (anti-HLA class II),[12] and then centrifuged over gradients of Percoll (Pharmacia),[16] and cells banding below 55% of Percoll collected. These cells did not proliferate to PHA, 0·3% v/v (Welcome), and contained $\leq 1\%$ of non-specific esterase-staining cells, $CD14^+$, large granular lymphocytes (LGL) and $CD19^+$ cells and $\geq 97\%$ were $CD3^+$. PBMC banding at 40–45% and 45–50% of Percoll gradients were used as a source of Mo and LGL, respectively. These cell preparations were mostly ($\geq 90\%$) $CD14^+$, positive for non-specific esterase reaction (40–45% Percoll), $CD56^+$ and exhibited LGL morphology (Percoll 45–50%). Irradiated (2000 rads) nylon-adherent cells were obtained as reported elsewhere.[12] B-cell-depleted mononuclear cells (BDMC) were obtained by complement-mediated lysis with mAb CD20 (B-C1) and CD24 (B-C2);[12] such cells contained $\leq 1\%$ $CD19^+$ cells. Purified tonsil B cells subfractionated with Percoll gradients, and purified $CD5^+$ B cells from patients with B-cell chronic lymphocytic leukaemia (B-CLL) were also obtained,[16,17] and contained $\leq 0{\cdot}5\%$ $CD14^+$ and $CD56^+$ cells, $\leq 2\%$ $CD3^+$ cells, and $\geq 93\%$ $CD19^+$ cells. Thymocytes were obtained from thymi of children undergoing cardiac surgery.[12] To obtain desialylated cells, these were resuspended (10×10^6 cell/ml) in 0·15 M NaCl (pH 5·9–6) without (mock-treated) or with (Nase-treated) 0·05 U/ml neuraminidase (Nase) from *Clostridium perfringens* (*Cl. perfringens*) (type VIII; Sigma), incubated at 37° for 45 min, and then washed twice in PBS.

Surface radioiodination, precipitation with lectins and SDS–PAGE

This was done as reported for mAb,[20] except that the buffer lysis also contained 1 mM $CaCl_2$ and 1 mM $MgCl_2$ and did not contain EDTA. Lysates were precleared with immobilized BSA (Sepharose-bound BSA), which was also used as a negative control of precipitation with immobilized (Sepharose- or agarose-bound) lectins.

Immunofluorescence cell surface staining

Direct or indirect immunofluorescence with mAb was done as described previously.[12,17] Three methods were used to assess PWM binding: (1) biotin-labelled PWM revealed by means of SA–FITC or SA–PE; (2) unconjugated PWM revealed by anti-PWM rabbit IgG, followed by FITC-conjugated anti-rabbit IgG goat antibodies; and (3) unconjugated PWM revealed by biotin-labelled anti-PWM rabbit IgG followed by SA–FITC or SA–PE. All lectins were used at a final concentration of 10 μg/ml. As negative controls, mouse IgG of unknown specificity (Becton Dickinson), either unconjugated or FITC-, PE- or biotin-labelled, was used. Incubation of cells with anti-PWM rabbit IgG was done in the presence of 10% heat-inactivated horse serum (Gibco BRL Europe, Merelbeke, Belgium) to avoid non-specific Fc receptor-dependent binding of rabbit IgG. Flow cytometry evaluation of fluorescent cells was done using a fluorescence-activated cell sorter (FACS) Star Plus or FACScan (Becton Dickinson); significant positive fluorescence differences between histograms for a given mAb or lectin were assessed on the basis of Kolmogorof-Smirnov statistics (LYSYS II Software; Becton Dickinson).

Cell cultures and proliferative assays

For proliferative assays, microcultures (in triplicate or quadruplicate) were done in a final volume of 0·2 ml, using 96-well plates as reported elsewhere.[12,16,17] The final concentration

of mitogens was: PWM (Sigma) 10 μg/ml; PNA (Sigma) 10–20 μg/ml; Sepharose-bound PWM and Sepharose-bound BSA, 25% v/v; PHA (Welcome) 0·3% v/v; and PMA (Sigma), 10 ng/ml. DNA synthesis was measured by [^{3}H]thymidine ([^{3}H]TdR) uptake during the last 16–18 hr of each culture period; results are expressed as the mean of triplicates or quadruplicates with SD values below 9% of the mean.[12,16,17] Viable PBT preactivated for 3 days with lectins in bulk cultures were obtained[12] and used for cell surface staining with mAb, HA–IL-2 binding assays and proliferative responsiveness to exogenous IL-2.

IL-2 determination and ^{125}I-IL-2 binding assay

IL-2 was measured with both the mouse CTLL-2 cell line[12] and with an enzyme-linked immunosorbent assay (ELISA) (Immunotech, Marseille, France). The ^{125}I-IL-2 binding assay was as reported elsewhere.[12]

RESULTS

PMA bypasses the Mo-dependency of PWM-induced T-cell mitogenesis

It still remains unknown whether PMA is able to bypass the Mo requirement for PWM to induce mitogenesis in human T lymphocytes, as is the case for Con A and PHA.[1,2] We investigated this because it has been suggested[10] that a Mo–PWM interaction could be strictly required for PWM to bind and activate T cells. If this is the case, PMA would be unable to bypass the Mo requirement. We found that PWM bypassed the Mo requirement for either soluble PWM or immobilized-PWM (Sepharose-bound PWM) or PHA to induce mitogenesis in PBT and thymocytes (Fig. 1). As expected,[12] IL-2 lacked a co-mitogenic effect for PWM-activated PBT and thymocytes, while it was co-mitogenic for the same cells activated with PHA. Irradiated autologous adherent cells were co-mitogenic for both PHA- and PWM-activated PBT. The synergy between PWM and PMA indicates that PWM is capable of interacting with the surface of T cells in the absence of Mo, delivering an activating signal that is complemented by PMA to result in T-cell mitogenesis. Therefore we then analysed the direct binding of PWM to blood T lympocytes, thymocytes and Mo by flow cytometry. The same was done for other leucocytes involved in PWM stimulation of PBMC, such as LGL and B cells, as it is known that PWM-stimulated natural killer (NK) cells can stimulate immunoglobulin secretion in resting human B cells,[21] and that PWM by itself initiates a signalling cascade in purified human splenic B cells.[22]

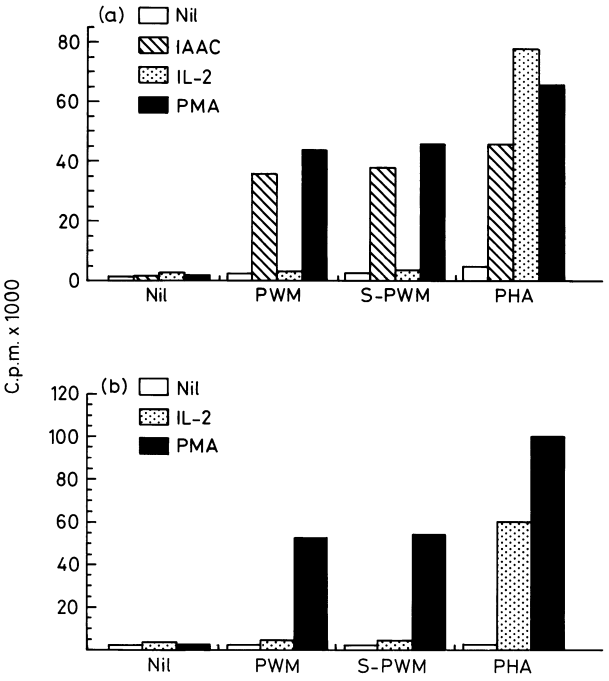

Figure 1. Proliferative response of PBT (1×10^5/flat-bottomed well) (a) and thymocytes (2×10^5/flat-bottomed well) (b) to soluble PWM (10 μg/ml), Sepharose-bound PWM (S-PWM, 25% v/v) and PHA (0·3% v/v) in the presence of culture medium alone (Nil) or equal numbers of irradiated autologous adherent cells (IAAC) or PMA (10 ng/ml) or IL-2 (10 U/ml). Representative independent experiments of three (thymocytes) and six (PBT) performed with cells from different donors are shown.

Reactivity of PWM with the surface of T and B cells, LGL and Mo

The binding of PWM to the surface of PBT was examined by using three methods (a, b, c) of increasing sensitivity (Fig. 2). As expected,[9,10] PWM was found to react much more strongly with Mo than with PBT (Fig. 2). The number of PBT that bound PWM increased with the higher sensitivity of the method used. Using the most sensitive methods (b and c), virtually all PBT bound PWM (Fig. 2). The higher reactivity of PWM with Mo than with PBT was corroborated by semi-quantitative precipitation experiments of lysates from cell-surface radio-iodinated Mo and PBT, which were adjusted to contain identical acid-precipitable c.p.m. (Fig. 3a). The reactivity of PWM with the surface of PBT was far weaker than that of Con A (compare the fluorescence histograms of the direct binding with FITC–Con A and indirect binding methods a and b for PWM; Fig. 2). This was also corroborated by semi-quantitative precipitation experiments (Fig. 3b). The reactivity of Mo with Con A was not much stronger than that of T lymphocytes (Fig. 2). The reactivity of FITC–PHA (data not shown) with PBT and Mo was essentially similar to that of FITC–Con A, as shown in Fig. 2.

Compared with PBT, thymocytes showed a higher reactivity with PWM and a lower reactivity with Con A (Fig. 2). The reactivity of LGL with PWM was as high as that of Mo (Fig. 4a). As expected,[23,24] PNA reacted with most thymocytes (Fig. 2) and most Mo (Fig. 4a), and completely failed to react with PBT (Fig. 4a). As with PWM, the reactivity of LGL with PNA was as high as that of Mo (Fig. 4a).

Figure 5 shows that the pattern of reactivity of PWM with leukaemic CD5$^+$ B cells and tonsil B cells was similar to that of PNA. As expected,[23, 24] PNA failed to bind mature resting B cells, as represented by leukaemic CD5$^+$ B cells, while it reacted with a significant proportion of tonsil B cells banding at 55–60% Percoll. This finding indicated the presence of *in vivo*-activated B cells[23,24] in this tonsil B-cell preparation, which is in accord with other data from our laboratory.[16]

As the pattern of reactivity of PWM with T and B

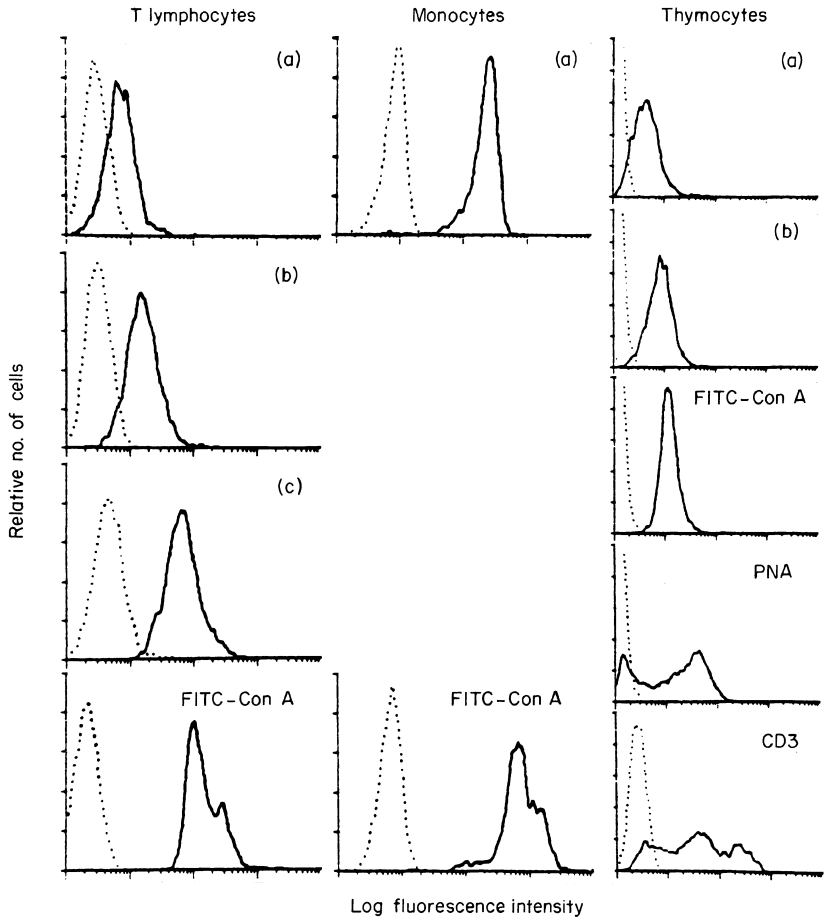

Figure 2. Reactivity of PWM with PBT, MO and thymocytes, using three different indirect immunofluorescence methods of increasing sensitivity: (a) biotin-labelled PWM, revealed by SA–FITC; (b) unconjugated PWM detected by anti-PWM rabbit IgG, followed by FITC-conjugated anti-rabbit IgG goat antibodies; (c) unconjugated PWM followed by biotin-labelled anti-PWM rabbit IgG, revealed by SA–FITC. PNA: biotin-labelled PNA. Lectins were used at 10 μg/ml. Discontinuous lines: negative controls. Representative experiments of four (thymocytes, Mo) and nine (PBT) performed with cells from different donors are shown.

lymphocytes, LGL, thymocytes and Mo is reminiscent of that of PNA, we examined whether desialylation of T and B lymphocytes increased their binding to PWM, as occurs for their binding to PNA.[23,24]

Desialylation of T and B lymphocytes strongly increases their PWM-binding capacity

As shown in Fig. 4b, Nase treatment of PBT strongly increased their PWM-binding capacity. As expected,[23,24] PNA became strongly reactive with Nase-treated PBT, whereas it completely failed to react with mock-treated PBT (Fig. 4b). Dasialylation of PBT did not affect their binding to FITC–Con A or FITC–PHA (data not shown). As can be seen in Fig. 5, desialylation of B lymphocytes also strongly increased their binding to PWM and PNA, this effect being more drastic in tonsil B cells (banding at 55–60% Percoll gradient) than in purified leukaemic $CD5^+$ B cells. The results shown in Figs 4 and 5 are representative experiments of two (Fig. 4a), three (Fig. 5a), five (Fig. 5b) and 10 (Fig. 4b) independent experiments performed with cells from different donors.

Desialylation of T lymphocytes bypasses the Mo requirement for PWM to induce T-cell mitogenesis and IL-2 secretion

Given the strong increase of the PWM-binding capacity of PBT after their desialylation, we investigated whether desialylation of these cells also influenced their responsiveness to PWM stimulation. As shown in Fig. 6a, PWM induced mitogenesis in Nase-treated PBT, and this response was not increased by exogenous IL-2, as occurs with the Mo-dependent T-cell mitogenesis induced by PWM in PBT plus irradiated autologous adherent cells (IAAC) (Fig. 1) or in BDMC (data not shown).[12] In accord with previous results (Fig. 1),[12] PWM failed to induce a proliferative response in mock-treated PBT even in the presence of exogenous IL-2, and the same occurred with PNA, an expected finding given the complete inability of this lectin to bind these cells. In desialylated PBT, PNA alone did not induce mitogenesis, but induced strong proliferative responsiveness to exogenous IL-2 (Fig. 6a). As expected, PHA alone failed to induce mitogenesis in both mock-treated and Nase-treated PBT, but induced a strong proliferative responsiveness to exogenous IL-2 in both PBT preparations (Fig. 6a).

As shown in Fig. 6b, the IL-2 production kinetics induced

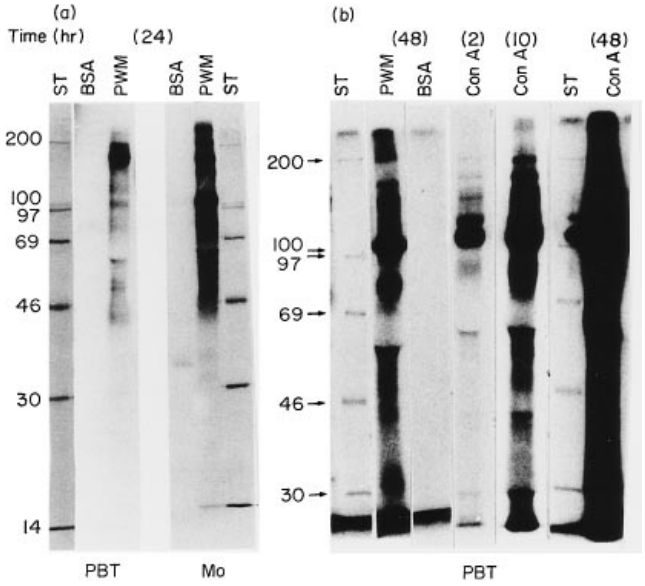

Figure 3. SDS–PAGE analysis of cell-surface proteins precipitated by (a) immobilized PWM or BSA in lysates of surface radio-iodinated Mo and PBT, which were adjusted to contain identical acid-precipitable radioactivity; (b) immobilized PWM, Con A or BSA in surface radio-iodinated PBT lysate. Autoradiography exposition times (hr) are indicated. ST, molecular size standards with their values in $MW \times 10^3$ indicated on the left.

by PWM, PHA and PNA in desialylated PBT were analysed and compared with that induced in untreated T lymphocytes plus Mo of the same individuals; for the latter purpose BDMC were used. Confirming our previous results,[12] the IL-2 levels (10–12 U/ml) in PWM-activated BDMC cultures were far higher than those (1·5–2 U/ml) found in PHA-activated ones, and the former remained unchanged even after 4 days of culture, while the latter were only detected during the first 24 hr. Remarkably, PWM, but not PHA, also induced IL-2 secretion in desialylated PBT, and the IL-2 levels were far higher (5–7 times) than those found in PWM-activated BDMC cultures, and persisted over time as occurred in the latter cultures. PNA failed to induce IL-2 production in both desialylated PBT and BDMC. The IL-2 levels found using the functional assay with CTLL-2 mouse cells (Fig. 6b) were confirmed by using a commercial ELISA kit (data not shown). The results shown in Fig. 6a,b, correspond to representative experiments of three and five, respectively, performed with cells from different donors.

PWM-activated desialylated T lymphocytes are unable to use IL-2 to sustain their mitogenesis and exhibit negligible HA–IL-2-binding capacity

The persistence over time of high IL-2 levels in the supernatants of PWM-activated desialylated PBT suggested a lack of IL-2 utilization, as occurs with Mo-dependent PWM-activated T lymphocytes.[12] Accordingly, the proliferative response of 3-day PWM-activated Nase-treated PBT did not increase when exposed to exogenous IL-2 (2·5, 5 and 10 U/ml), while that of 3-day PHA-activated ones showed a linear dependency on the IL-2 dose (Fig. 6c). These data, which were confirmed in three additional experiments with cells from different donors, indicate that, as occurs with Mo-dependent PWM-activated T cells,[12] PWM-activated desialylated PBT lack HA–IL-2 binding capacity. This was clearly demonstrated by analysing in parallel the HA–IL-2 binding capacity of PWM-activated desialylated T cells and PWM-activated BDMC of the same individuals (Fig. 7) The results found with PHA- and PWM-activated BDMC confirm our previous results.[12] As expected,

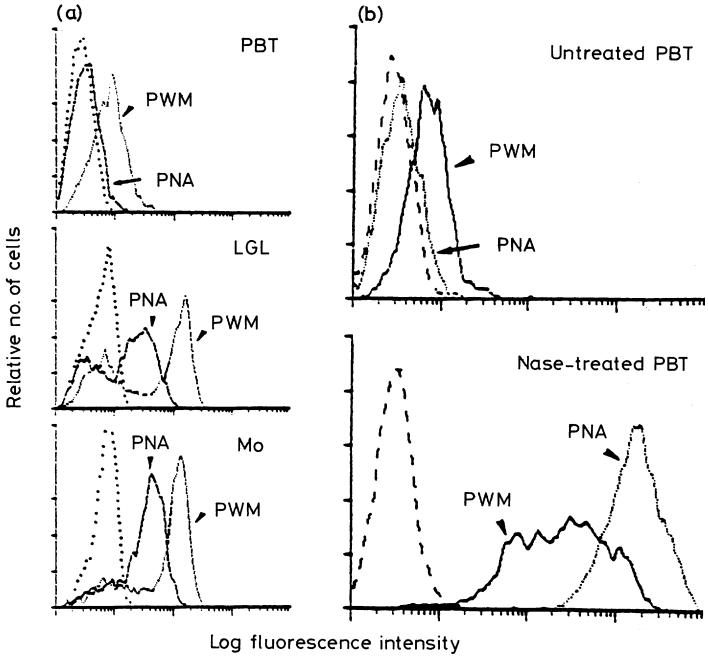

Figure 4. Reactivity of biotin-labelled PWM [method (a) in Fig. 2] and biotin-labelled PNA with freshly PBT, Mo and LGL (a), and with mock-treated and Nase-treated PBT (b). Lectins were used at 10 μg/ml. Representative experiments of two (a) and 10 (b) performed with cells from different donors are shown. Dotted/dashed lines: negative controls.

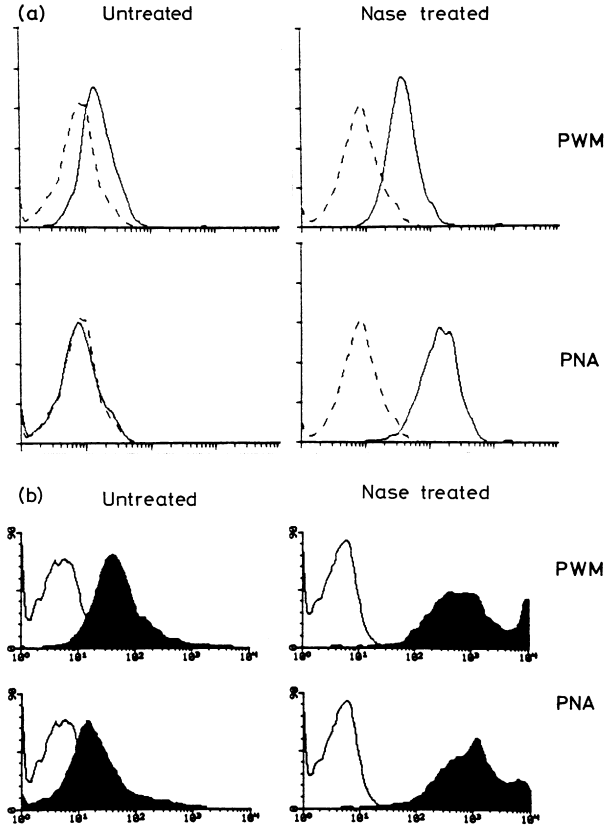

Figure 5. Reactivity of biotin-labelled PWM [method (a) in Fig. 2] and biotin-labelled PNA with mock-treated or Nase-treated (a) purified leukaemic CD5$^+$ B cells and (b) purified tonsil B cells banding at 55–60% Percoll gradient. *y*-axis, relative number of cells; *x*-axis, fluorescence intensity (log). Representative experiments of three (leukaemic CD5$^+$ B cells) and five (tonsil B cells) performed with cells from different donors are shown. Dashed (a) and non-shaded (b) curves: negative controls.

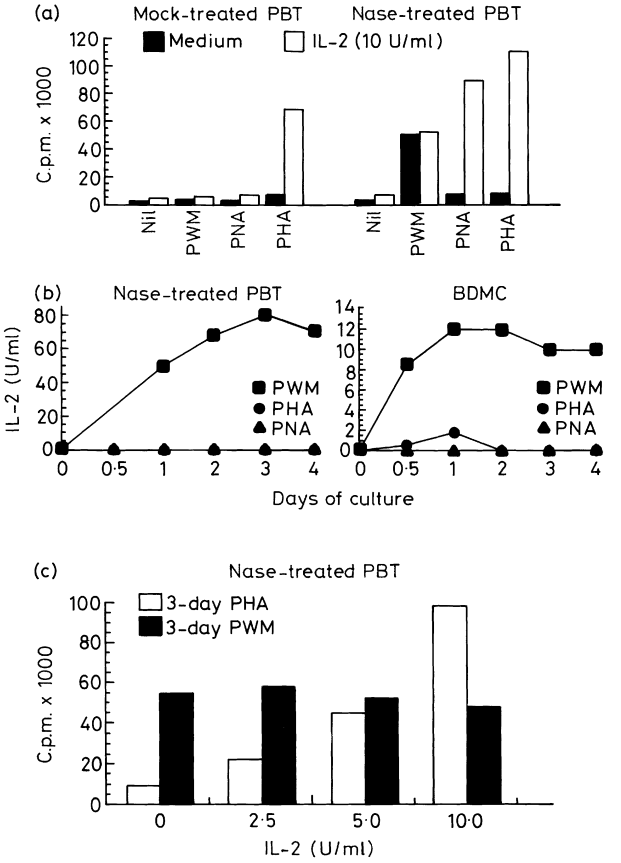

Figure 6. (a) Capacity of PWM (10 μg/ml), PNA (20 μg/ml) and PHA (0·3%, v/v) to induce a proliferative response in mock-treated and Nase-treated PBT in the absence or presence of IL-2 (10 U/ml). Culture conditions and expression of results as in Fig. 1. (b) IL-2 levels (measured by a functional assay with the CTLL-2 mouse T-cell line) in the supernatants of Nase-treated Nase-treated PBT and BDMC before and after several culture periods with PWM, PNA and PHA. (c) Proliferative responsiveness to exogenous IL-2 of 3-day preactivated T cells ($0{\cdot}5 \times 10^5$/round-bottomed well) generated by stimulation of Nase-treated purified T lymphocytes with either PHA (3-day PHA) or PWM (3-day PWM).

PNA failed to induce HA–IL-2 binding capacity in BDMC, while in desialylated PBT this lectin induced a HA-IL-2 binding capacity similar to the one induced by PHA (Fig. 7).

The HA IL-2 binding inability of PWM-activated desialylated PBT was concomitant with a reduced expression of IL-2R α chain compared with that of PHA-activated PBT (Fig. 8), a finding also found on Mo-dependent PWM-activated T cells (data not shown).[12] This finding was more marked with CD25 mAb MAR108, than with CD25 mAb from Becton Dickinson (IL-2R) (Fig. 8). MAR108 mAb, is a potent inhibitor of IL-2-dependent proliferation.[12,19] Note that PHA-activated desialylated PBT did not produce detectable IL-2, while very high IL-2 levels (60–80 U/ml) were present in cultures of 3-day PWM-activated PBT; therefore, these data clearly indicate that the IL-2 produced by the latter cells fails to up-regulate[1–3] their IL-2R α subunit expression, a finding corroborating the above data on the inability of these cells to bind and utilize IL-2. The IL-2R β expression detected by TU27 mAb[18] on PWM-activated desialylated T cells compared with that of PHA-activated T cells was also reduced, a finding also occurring in Mo-dependent PWM-activated T cells (data not shown).[12] The expression of IL-2R α and β chains induced by PNA on desialylated PBT (data not shown) was similar to that shown for PHA-activated PBT in Fig. 8. The reduced IL-2R chain expression on PWM-activated desialylated PBT was not due to an insufficient activation, since other activation-related parameters such as expression of CD98 (4F2) antigen (data not shown) or increased cell size (Fig. 8), were similar for both PHA-activated and PWM-activated desialylated PBT. The results shown in Fig. 7 and Fig. 8 are representative experiments of two and three, respectively, performed with cells from different donors.

DISCUSSION

The aim of this study was to gain insight on whether the impaired functional IL-2R expression on Mo-dependent PWM-activated T cells arises in T cells themselves or is due to Mo-derived negative influences (see the Introduction). As a first approach, the interaction of PWM with the surface of T lymphocytes was examined and compared with that of classical lectins inducing Mo-dependent IL-2-mediated T-cell proliferation, Con A or PHA, as well as with PNA, as this information

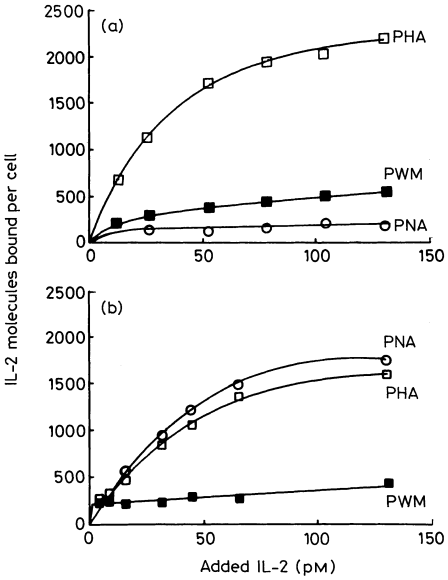

Figure 7. HA IL-2 binding capacity (*y*-axis, expressed as molecules/cell) in the presence of pM concentrations of ^{125}I-labelled IL-2 (*x*-axis), of (b) Nase-treated PBT activated for 3 days with PWM, PNA and PHA, compared with that of (a) BDMC of the same individual activated for 3 days with the same lectins. The results shown for PNA-activated BDMC were indistinguishable from those found in unstimulated cells (data not shown). A representative experiment of two with cells from different donors is shown; data correspond to the mean of duplicate determinations with SD values below 4% of the mean.

was lacking and is relevant. These results show, for the first time, that Nase treatment of T and B lymphocytes strongly increases their PWM-binding capacity, in a manner reminiscent of PNA.[23,24] Remarkably, desialylation of T lymphocytes replaces Mo for PWM to induce mitogenesis and IL-2 secretion, and such secreted IL-2 levels are even higher (5–7 times) than those found in Mo-dependent PWM-activated T lymphocytes. However, similarly to the latter, PWM-activated desialylated purified T lymphocytes exhibited negligible HA–IL-2 binding capacity and an inability to utilize the IL-2 they produce. These data for PWM-activated desialylated PBT clearly indicate that the impaired expression of functional IL-2R in PWM-activated T cells is due to intracellular events arising in PWM-activated T cells themselves. That the results in PWM-stimulated desialylated PBT were not due to desialylation itself is indicated by data obtained in the same cells stimulated with PHA or PNA.

IL-2 has lectin properties with a sugar specificity for N,N-diacetylchitobiose,[25] which has been also implicated for the sugar specificity of PWM (vide infra). However, PWM is unlikely to act by interfering with the binding of IL-2 to IL-2R, since the IL-2 carbohydrate binding site is different from the IL-2R binding site.[25] In addition, we have demonstrated that the binding of PWM on the surface of PHA-preactivated T cells does not affect the detection of HA IL-2R and IL-2R α and β chains.[12]

Present data demonstrate that the binding of PWM to T lymphocytes is far weaker than that of classical lectins inducing Mo-dependent IL-2-mediated T-cell proliferation, Con A or PHA. Such binding, however, is sufficient to ligate T cell-surface molecules capable of transducing activation signals, which are complemented by PMA to induce DNA synthesis in PBT and thymocytes. This finding does not support that a PWM–Mo interaction is required for PWM to bind and initiate the activation of T lymphocytes.[10]

The sugar specificity of PWM is not clearly defined, and both branched poly N-acetyllactosamine chains as well as the N,N′-diacetylchitobiose moiety have been implicated (reviewed

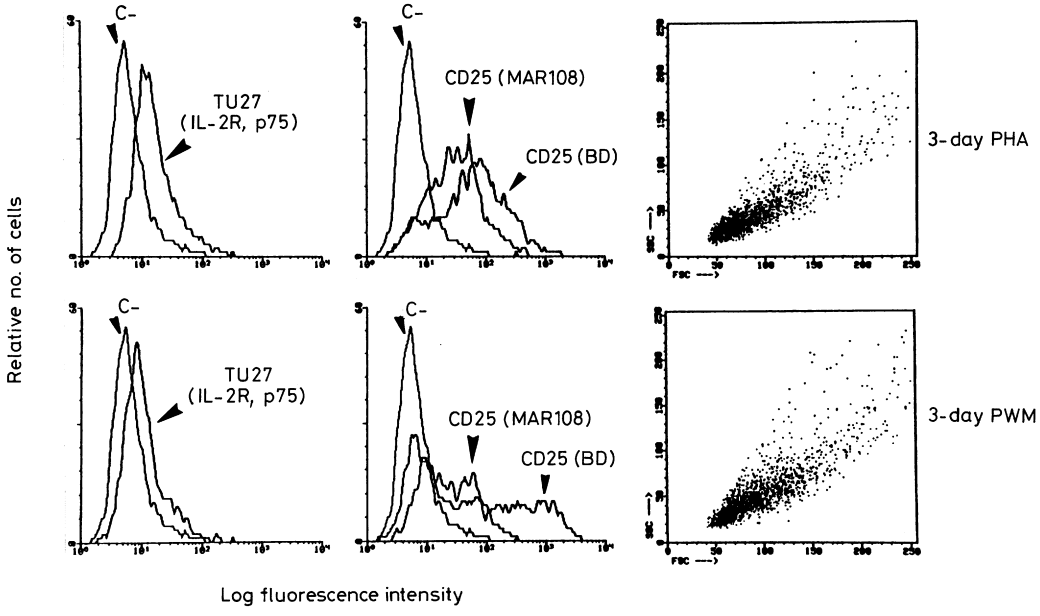

Figure 8. Comparison of IL-2R α and IL-2R β (p75) chain expression by Nase-treated PBT preactivated for 3 days with either PWM or PHA. The results with PNA (not shown) were similar to those shown for PHA. The side and forward scattering dot plots of cells are also shown. IL-2R α chain was detected by CD25 mAb MAR108[19] and IL-2R from Becton Dickinson (BD); IL-2R β was detected by TU27 mAb.[18] C-, negative controls.

in ref. 26). Desialylation can facilitate exposition of poly N-acetyllatosamine, as occurs for I/i antigens which comprise branched (I) or linear (i) poly N-acetyllactosamine chains.[27] Of note, other workers have reported that the binding of PWM to human B cells was not modified by desialylation.[8] This discrepancy with the present results presumably reflects the use of a different B-cell preparation and Nase source, as in that study[8] a blood B-cell fraction was used, the contamination of Mo, which is usually high, was not indicated, and it utilized Nase (200 mU/ml) from *Arthrobacter ureafaciens*, the effectiveness of which was controlled by the *Lymax flavus* lectin binding, and it was not very clear.[8] In the present study highly purified leukaemic $CD5^+$ B cells and tonsil B cells (55–60% Percoll) with undetectable Mo or LGL were used, and the effect of Nase (50 mU/ml) from *Cl. perfringens* (type VIII; Sigma) was clearly demonstrated by the PNA binding. Nase from *Cl. perfringens* or *Vibrio cholera* is commonly used to desialylate lymphoid or red blood cells.[23,24,27]

The fact that desialylation strongly increases the PWM-binding by T lymphocytes and replaces Mo for PWM to induce T-cell mitogenesis, suggest that one of the main Mo accesory functions for this lectin could be to provide a source of Nase activity, which would favour the strong binding of PWM to surface molecules relevant for T-cell activation. A Nase activity increase is an early event in the activation of mouse splenocytes.[28] These data may suggest that during infections an increased *in vivo* Nase activity could result from pathogen-mediated activation of Mo/macrophages and/or from the pathogen's own Nase, a virulence factor shared by many pathogens. The resulting desialylation of T and B lymphocytes could promote their binding to, and polyclonal activation by, pathogen-derived compounds acting as PWM-like lectins and favour a T-cell-dependent polyclonal differentiation of B cells, as PWM do. Such a possibility could help explain the polyclonal hypergammaglobulinaemia exhibited by patients with chronic infections.

Commitment of T cells for the IL-2-dependent proliferative pathway involves induction of IL-2 secretion and induction of IL-2R expression, these events being differently regulated, as indicated by the fact that the former has far more stringent activation signal requirements, and is more easily inhibited, than the latter.[1–3] Moreover, while regulation of IL-2 production is mainly transcriptional or post-transcriptional at the level of transcript stability,[1–3] the existence of a translational control for IL-2R α chain has been found in the murine system.[29] The PWM-induced activation of T cells clearly unravels the existence of intracellular mechanisms that can impair the expression of functional IL-2R while failing to affect IL-2 production and secretion. The effects of the immunosuppressant rapamycin[30] and a *Trypanosoma cruzi*-derived factor (reviewed in ref. 31) also support this notion. The presence of a similar immunosuppresant contaminating PWM preparations should be considered, given that from the pokeweed plant ribosome-inactivating proteins are also extracted.[32] Whichever the case, the strong binding of PWM on desialylated T cells and the Mo-independent character of PWM-induced activation in desialylated T lymphocytes provides a simplified experimental system, without contaminating monocytes, suitable for facilitating the search for the T-cell surface molecules that interact with PWM and mediate T-cell activation, as well as the search for intracellular molecular events capable of impairing the expression of functional IL-2R without affecting IL-2 production and secretion.

ACKNOWLEDGMENTS

This study was supported by a grant from 'Fondo de Investigaciones de la Seguridad Social, Ministerio de Sanidad y Consumo' (FISS 93/0718). Many thanks are due to Dr K. Sugamura (Tohoku, Japan), Dr F. Sánchez-Madrid (Madrid, Spain) and Dr M. López-Botet (Madrid, Spain) for providing mAb (see the Materials and Methods) and to Dr J. Vives Puiggròs (Barcelona, Spain) for helpful suggestions in reviewing the manuscript.

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