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ORIGINAL ARTICLE

The effect of anti-lymphocyte serum on subpopulations of blood and tissue leucocytes: possible supplementary mechanisms for suppression of rejection and the development of opportunistic infections

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Summary

Xenogeneic anti-lymphocyte serum (ALS) remains a major reagent for immunosuppression in clinical practice, but mechanisms of action and risks of opportunistic infection have not been considered in the context of innate immunity and its role in immune responsiveness. Rabbit anti rat ALS was administered intraperitoneally. Blood was taken for flow cytometry to establish absolute counts of leucocyte subsets. Tissues were harvested for immunohistology to evaluate interstitial dendritic cells and tissue macrophages. At day 2 of ALS therapy, T cells are completely depleted, as anticipated. B cells are undiminished and form approximately 90% of blood leucocytes. Monocytes and natural killer (NK) cells are substantially (approximately 80%), but not completely, depleted, and there is a trend for diminished numbers of putative dendritic cells. Neither interstitial dendritic cells nor tissue macrophages in heart are affected. The results at day 7 were very similar to day 2. Substantial depletion of blood monocytes and NK cells might attenuate the innate immune system, and represent a possible supplementary mechanism (in addition to T cell depletion) for suppression of rejection. It might be of particular importance in reducing defences against infections. Monitoring these parameters could be of clinical value.

Introduction

The immunosuppressive properties of xenogeneic anti-lymphocyte sera (ALS) were discovered in the 1960s [1], and ALS came into increasing clinical prominence from ca 1980. It remains today a major reagent for immunosuppression in clinical transplantation [2].

The effectiveness of ALS is unquestioned. However, its mechanism of action has not been considered in the light of the role of innate immunity in the adaptive immune response [3,4]. The most obvious effect of ALS is T lymphocyte depletion [5]. If this is complete, it is a *sufficient* mechanism for its immunosuppressive effects. However, only very small numbers of T cells are able to evoke effective allograft rejection [6]. In clinical practice, T cell levels in ALS-treated patients are maintained below low but specified levels. Of particular importance, the only reported clinical study to attempt control of rejection purely by T cell depletion (using the OKT3 monoclonal antibody) was not successful, because of the development of strong anti-mouse immunoglobulin antibodies towards the end of the first week of therapy [7].

Another area of potential clinical importance is the better current understanding of the role of innate immunity in the defence against microbial infections. Innate immunity frequently represents the first line of defence against infection by organisms as diverse as viruses [e.g. 8,9], bacteria [e.g. 10,11], protozoa [e.g. 12,13] and fungi [e.g. 14]. Impairment of innate immunity might therefore have a disproportionate effect on the body's defences against opportunistic infections, as it affects both first and second line defences.

We have studied the *in vivo* effects of rabbit anti rat ALS, using whole blood flow cytometry and immunohistology, primarily to see if there are possible supplementary mechanisms (in addition to T cell depletion) contributing to the immunosuppressive effects of ALS. We were also interested to see if these studies might help to clarify the blood precursor of the interstitial dendritic cell [15]. It is of interest in this regard that natural killer (NK) cells and macrophages appear to play a role in allograft rejection [16,17]. An effect of ALS on the leucocytes of the innate immune system could be of importance for suppression of the recipient's immune system, in relation both to allograft rejection and the development of opportunistic infections. In addition, in the particular context of transplantation, an effect of ALS on interstitial dendritic cells *in the donor organ* [15,18] could influence allograft immunogenicity.

Materials and methods

Rats

Adult male rats of the DA ($RT1^{av1}$) and LEW ($RT1^{l}$) strains (Harlan UK Ltd., Bicester, UK), were used between 12 and 16 weeks of age.

Rabbits

New Zealand White rabbits, weighing 2–3.5 kg were from Harlan UK Ltd.

Anti-thymocyte serum

A single cell suspension was prepared from the thymuses of DA rats, and contaminating erythrocytes were lysed using erythrocyte lysis buffer [19]. Rabbits received an intravenous injection of 1×10^8 thymocytes in 2 ml of phosphate buffered saline (PBS), and a booster injection 2 weeks later of 5×10^7 thymocytes. They were bled of 30 ml blood 10 days after the boost injection, and exsanguinated 4 days later. The sera were pooled, heat inactivated at 56 °C for 30 min, and stored in 1.1 ml aliquots at −40 °C.

Separation of leucocytes from whole blood

Eight millilitre of heparinized rat blood was layered on to 5 ml of Histopaque 1077 (Sigma-Aldrich, Poole, Dorset, UK), and centrifuged at 770 *g* for 30 min at room temperature. The leucocyte layer was removed and washed three times in PBS by centrifugation at 400 *g* for 5 min.

Irradiation

Rats were given 1000 rads of whole body γ irradiation at the rate of approximately 200 rads/min from a cobalt source (Mainance GP1200, Mainance Engineering, Portsmouth, UK), and were killed 4 days later by exsanguination.

Monoclonal antibodies

Mouse antibodies to rat leucocyte antigens, directly conjugated with either fluorescein isothiocyanate (FITC) or R-phycoerythrin (RPE), were obtained from Serotec Ltd, Oxford, UK. A mouse antibody to rat CD45, directly conjugated with R-PE:cyanine-5 (PE:Cy5) (Biocarta US, San Diego, CA, USA) was used as a third colour for the positive identification of leucocytes. The F15-42-1 IgG1 mouse antibody to human Thy-1 (CD90) [20], directly conjugated with either FITC or RPE, was used as a negative control. Details as follows:

1 MRC OX1 IgG1 antibody (PE:Cy5) to CD45, present on all rat leucocytes [21]
2 IF4 IgM antibody (FITC) to CD3 [22]
3 R73 IgG1 antibody (FITC) to the alpha/beta T cell receptor [23]
4 V65 IgG1 antibody (FITC) to the gamma/delta T cell receptor [24]
5 MRC OX33 IgG1 antibody (FITC and RPE) to CD45RA on B cells [25]
6 ED1 IgG1 antibody (FITC) to monocytes [26]
7 ED2 IgG1 antibody (FITC) to CD163 on tissue macrophages [26]
8 MRC OX62 IgG1 antibody (RPE) to rat dendritic cells [27]
9 10/78 IgG1 antibody (FITC) to CD161 on NK cells [28]
10 MRC OX6 IgG1 antibody (FITC and RPE) to RT1-B MHC class II antigens [29]

Fluorescein labelled goat anti rabbit immunoglobulin

Affinity purified goat $F(ab')_2$ anti rabbit $F(ab')_2$ was prepared and fluorescein labelled as previously described [30].

Immunofluorescence staining

Immunofluorescence staining was performed within 1 h of blood collection. In the standard protocol, saturating amounts of directly conjugated monoclonal antibodies (10 µl of each antibody at 100 µg/ml) were added to 100 µl of heparinized blood and incubated for 30 min at

room temperature. Two millilitre of the erythrocyte lysis buffer was then added and incubated for 10 min. The cells were washed twice by centrifugation at 400 *g* for 5 min in PBS and then resuspended in 0.5 ml of 1% paraformaldehyde in PBS. The cells were stored in the dark at 4 °C and analysed within 24 h.

For ED1 (primarily an intracellular antigen), 100 μl blood samples were first incubated with 10 μl of the antibody to CD45 for 30 min. One hundred microlitre fixation medium (Reagent A–Leucoperm; Serotec Ltd) was then added, followed by 15 min incubation. Two millilitre of PBS was added and the sample was washed once by centrifugation at 400 *g* for 5 min. The cell pellet was resuspended in 100 μl of permeabilization medium (Reagent B-Leucoperm; Serotec Ltd), which permeabilizes leucocytes and also lyses red cells. Ten microlitre of ED1 antibody at 100 μg/ml was then added to the sample, and incubated at room temperature for 30 min. Two millilitre of PBS was added and the sample was washed once by centrifugation at 400 *g* for 5 min. The cell pellet was then resuspended in 0.5 ml of 1% paraformaldehyde in PBS, and processed as above.

For detection of ALS on purified blood leucocytes, 25 μl of cells at 4×10^7 /ml in PBS were incubated with an equal volume of fluorescein-labelled goat anti rabbit immunoglobulin (GAR) at 20 μg/ml for 30 min, washed twice by centrifugation, and then processed as above.

Flow cytometry

Samples were analysed using a FACSCalibur (Becton Dickinson, Oxford, UK) equipped with an air-cooled argon-ion laser and detectors for forward scatter (FSC), 90° scatter (side scatter, SSC), and for FL1 (530 nm), FL2 (585 nm) and FL3 (650 nm) fluorescence emissions. FITC, RPE and PE:Cy5 were detected on the FL1, FL2 and FL3 channels respectively. Acquisition and data analysis was performed using CellQuest software (Beckton Dickinson). Fluorescence overlap was compensated electronically. A minimum of 5000 events was acquired and stored for each analysis.

Leucocytes were identified on a dot plot of SSC versus CD45 fluorescence (FL3), and mononuclear cells were electronically gated for further analysis. Three-colour analysis was used to identify T cells (FITC anti CD3) and B cells (RPE anti CD45RA) within the CD45 (PE:Cy5) positive mononuclear cell population. Two-colour analysis was used to identify monocytes (FITC anti ED1) and NK cells (FITC anti CD161) within the CD45 positive mononuclear cell population. Three-colour analysis with a cocktail of FITC-labelled antibodies (anti CD3, CD45RA, CD161, ED1, TCR gamma/delta) was used to identify RPE-labelled OX62 positive cells lacking lineage specific markers (putative dendritic cells) within the CD45 positive mononuclear cell population.

Absolute blood leucocyte counts were obtained using Flow-Count™ Fluorospheres (Beckman Coulter, High Wycombe, Bucks, UK). The Flow-Count (Direct) method involves mixing a known volume and concentration of Flow-Count Fluorospheres with an equal volume of whole blood. In our studies, 100 μl of fluorospheres at a specified concentration was added to the paraformaldehyde fixed leucocytes (which were derived from 100 μl of whole blood), just prior to flow cytometry. Absolute blood leucocyte counts were then determined using the following formula:

$$\text{Cells per μl of whole blood} = \frac{\text{Number of cells counted}}{\text{Number of fluorospheres counted}} \times \text{Fluorosphere concentration}$$

A minimum of 1000 fluorospheres was acquired for each analysis.

Immunohistology

Tissue samples were harvested from exanguinated rats and immediately frozen in liquid nitrogen. Sections of 6 μm were cut, air dried for 2 h at room temperature and incubated for 30 min with saturating concentrations of FITC anti CD163 (ED2) to detect tissue macrophages and FITC anti MHC class II (OX6) to detect interstitial dendritic cells [15,18]. FITC anti human CD90 was used as the negative control. Sections were washed twice in PBS and mounted in Vectashield Mounting Medium for fluorescence microscopy (Vector Laboratories, Inc. Bulingame, CA, USA).

Statistical analysis

The student's *t*-test was used, and groups were considered significantly different if P was <0.05 in two tailed tests.

Results

Baseline transplantation studies

To confirm that the batch of ALS analysed in this study was immunosuppressive, it was tested in the DA to LEW cardiac allograft model. DA hearts were transplanted heterotopically to the aorta and inferior vena cava of LEW recipients, using standard microsurgery techniques. In three untreated recipients, the heart allografts ceased beating on days 6, 7, and 7 days. In three recipients given 1 ml of ALS intraperitoneally on the day of grafting and on days 1, 3 and 5, all allografts were beating strongly and normally for >21 days.

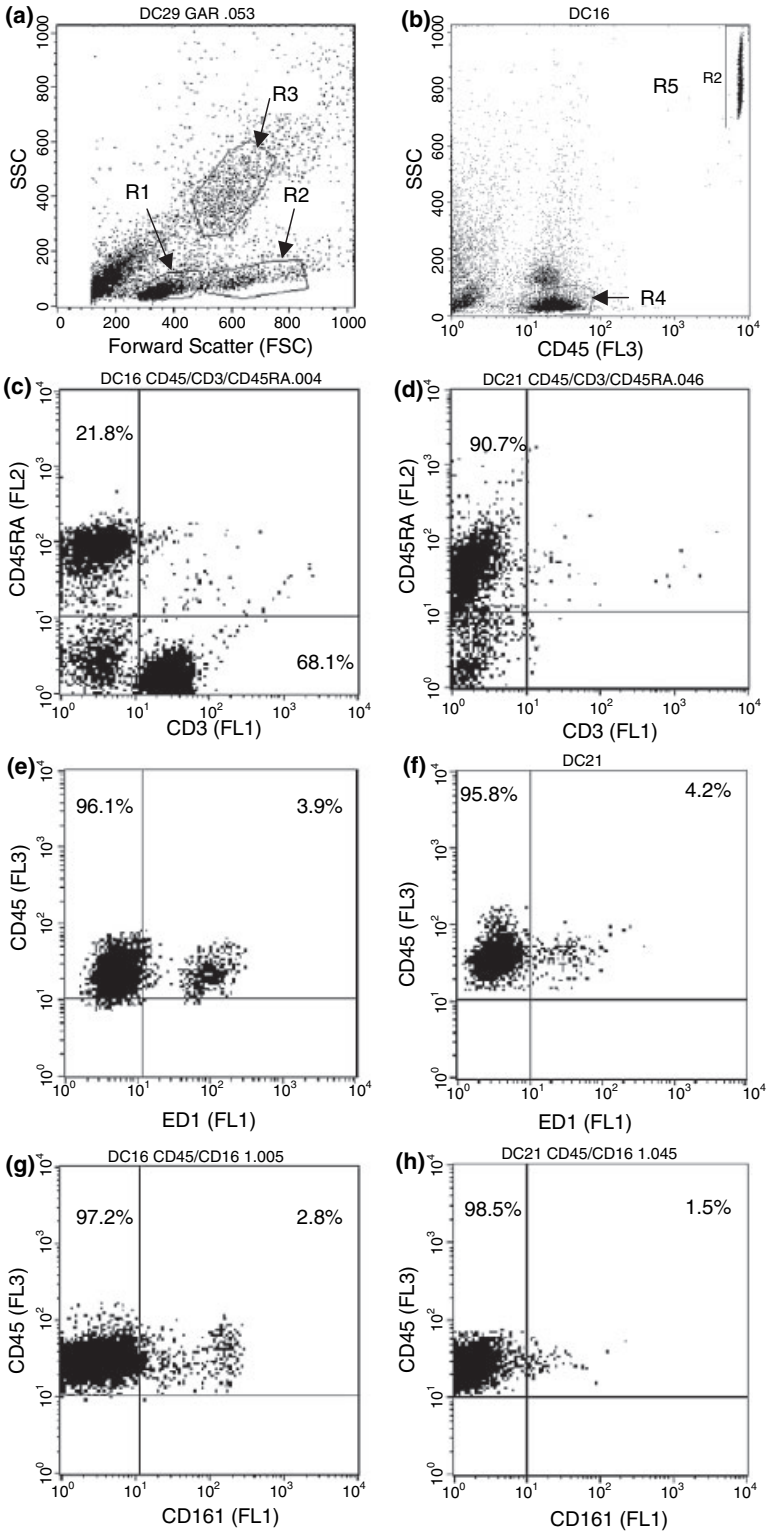

Figure 1 Whole blood flow cytometry analysis. Typical scatter parameters for a normal DA strain rat are shown in (a) and (b). (a) Forward (FSC) versus side (SSC) scatter. Gated areas R1, R2 and R3 correspond to lymphocytes, monocytes and granulocytes. (b) Side scatter versus CD45 fluorescence. Gated area R4 corresponds to mononuclear cells (lymphocytes and monocytes). Fluorescent microspheres in gated area R5. (c, d) Three colour analysis of the CD45 positive R4 gated cells, showing T and B cell markers in a normal rat (c) and a rat given ALS on days 0, 1, 3 and 5, and blood taken at day 7 (d). (e–h) Two colour analysis of R4 gated cells showing CD45 fluorescence versus monocyte and NK cell markers in a normal rat (e, g) and an ALS-treated rat (f, h).

Baseline flow cytometry studies on normal DA strain rat blood

Whole blood fluorescence flow cytometry enables a precise measurement of leucocyte populations without the risk of losses, especially selective losses, during leucocyte purification from blood. A typical dot plot of FSC versus SSC is given in Fig. 1a. The R1, R2 and R3 regions have previously been reported as containing lymphocytes, monocytes and granulocytes respectively [31], and we have confirmed this using the cell-specific antibodies listed in the Methods section (data not shown).

Figure 1b shows a dot plot of SSC versus CD45 fluorescence as a pan-leucocyte marker. This has the advantage of more clearly and definitively distinguishing leucocytes from the nonleucocyte debris, primarily red cell ghosts, and was therefore used in this study. Monocytes are not distinguished from other mononuclear cells, and fall in the R4 region, but this is not a problem because of cell-type specific monoclonal antibodies. The R5 region contains the fluorospheres for quantitative analysis.

Analysis of T cells, B cells, monocytes and NK cells in a typical blood sample, are shown in Fig. 1c, e and g. Figure 1c is a three colour analysis showing B cells and T cells in the CD45 positive R4 region. Figure 1e and g show monocytes and NK cells respectively in two colour analyses of R4 gated cells, plotting CD45 fluorescence versus the cell-specific marker.

Baseline flow cytometry studies on blood leucocytes in ALS treated rats

Before examining ALS treated rats, it was important to check that the ALS does not competitively inhibit the binding of the lineage-specific monoclonal antibodies. To test this, we initially added 1 ml of heat-inactivated ALS to 9 ml of whole blood, with the idea of proceeding with the addition of the directly conjugated lineage-specific monoclonal antibodies as the second step. This caused complete lysis of all blood leucocytes (see later), in spite of the fact that the ALS had been heat inactivated. As this was probably because of the presence of rat complement in the blood, we purified leucocytes from blood for this analysis. The leucocytes were incubated in a one in 10 dilution of the ALS for 30 min prior to flow cytometry. This did not result in leucocyte lysis, consistent with the idea that ALS-mediated lysis was dependent on rat complement. Pre-incubation with the ALS did not block any of the monoclonal antibodies tested: CD3, CD45, CD45RA, ED1 or CD161 (data not shown).

Five rats were given 1 ml of ALS intraperitoneally on days 0, 1, 3 and 5, and exsanguinated on day 7. T cells were virtually totally eliminated, and B cells comprised approximately 90% of blood mononuclear cells (Fig. 1d). Monocytes (Fig. 1f) and NK cells (Fig. 1h) were still present, but as the percentages were either normal or less than normal in the face of T cell depletion, it was likely that this would

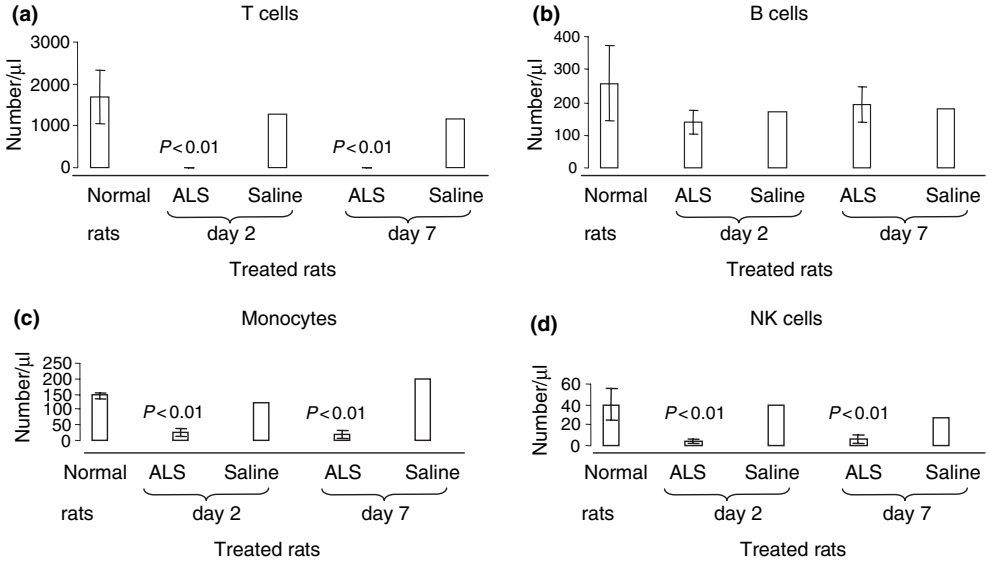

Figure 2 Quantitative analysis of blood leucocytes in normal rats and rats treated with ALS. Rats were given ALS on days 0, 1, 3 and 5, and blood was taken at day 2 or day 7. Absolute levels of leucocyte subsets in whole blood were calculated using fluorescent microspheres, as described in the Methods. The results show T cell, B cell, monocyte and NK cell numbers in a–d respectively in normal rats ($n = 5$), rats given ALS on days 0 and 1 and analysed on day 2 ($n = 4$), and rats given ALS on days 0, 1, 3 and 5 and analysed at day 7 ($n = 5$). Control rats ($n = 2$ for day 2, $n = 2$ for day 7) were given saline instead of ALS. The results are mean ± SE, except for saline treated controls, where no error bars are given. The *P*-values are for ALS-treated rats compared with saline-treated plus normal rats.

represent a depletion in absolute numbers. Four rats received ALS on days 0 and 1, and were exsanguinated on day 2. The results were very similar to those seen at day 7.

Absolute blood counts

Absolute levels of blood leucocyte subpopulations have not been reported in the normal rat, so far as we have been able to find. We evaluated five normal DA male rats, and the results are given in Fig. 2 under 'normal rats'. The values (mean ± 1 SE) were 1682 ± 283 T cells, 257 ± 51 B cells, 145 ± 4.9 monocytes and 40 ± 6.7 NK cells per microlitre of blood. These values are similar to those seen in man and mouse.

In the ALS treated rats, T cells are confirmed to be essentially totally eliminated (Fig. 2a), but there is no effect of the ALS on B cells (Fig. 2b). There is a substantial (approximately 80%) although not complete depletion of monocytes (Fig. 2c) and NK cells (Fig. 2d).

The persistence of B cells, monocytes and NK cells could be a consequence of insufficient ALS dosage, such that the surviving blood leucocytes were not coated with antibodies. Blood leucocytes from the ALS treated rats were therefore checked at day 7 for coating with rabbit antibodies. Because normal rabbit immunoglobulins in the blood of the ALS treated rats would block the goat antibodies to rabbit Ig, whole blood flow cytometry was not possible. The leucocytes were therefore purified from blood prior to flow cytometry. The results are given in Fig. 3, and demonstrate that lymphocytes and monocytes were all coated with rabbit Ig.

Putative blood dendritic cells in normal and ALS treated rats

Studies in normal DA rats

The OX62 antibody has been evaluated on single cell suspensions of organized lymphatic tissues [e.g. 32] and on tissue sections [e.g. 33], but blood leucocytes do not appear to have been studied. The results of a typical analysis of blood from a normal DA rat are given in Fig. 4. We were surprised to find a large cohort of OX62 positive cells in blood, as illustrated in Fig. 4b. A cocktail of fluorescein-labelled lineage-specific antibodies (to CD3, CD45RA, CD161, ED1 and TCR gamma/delta receptor) was used to identify a population of OX62 positive cells without established lineage markers. These amounted to approximately 3% of blood leucocytes (Fig. 4c). If antibody to MHC class II antigens was added to the cocktail, it can be seen that most of these cells were MHC class II positive (Fig. 4d).

Studies in ALS treated rats

The CD45 positive blood leucocytes which expressed OX62 but not lineage-specific markers were taken as putative dendritic cells. Absolute numbers of these putative

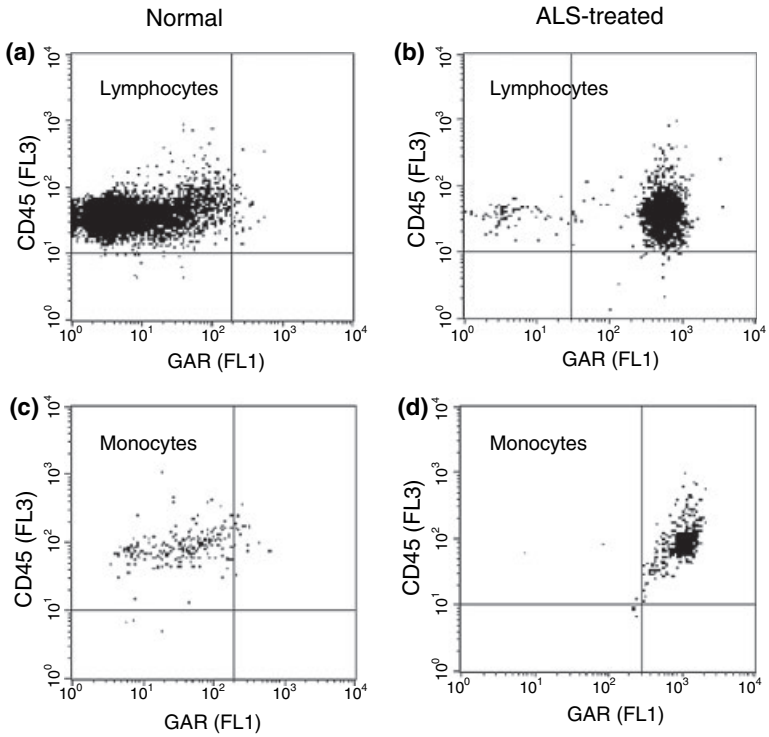

Figure 3 Coating of blood leucocytes with rabbit antibody. Rats were given ALS on days 0, 1, 3 and 5, and bled at day 7 for flow cytometry analysis. Leucocytes from normal rats (a, c) or ALS-treated rats (b, d) were incubated with fluorescein labelled goat anti rabbit Ig. The PE:Cy5 labelled antibody to rat CD45 was included in all samples. The cells were gated by forward and side scatter (as in Fig. 1a) and the R1 gate (a, b), and R2 gate (c, d), corresponding to lymphocytes and monocytes respectively, were analysed. Two colour analyses are shown.

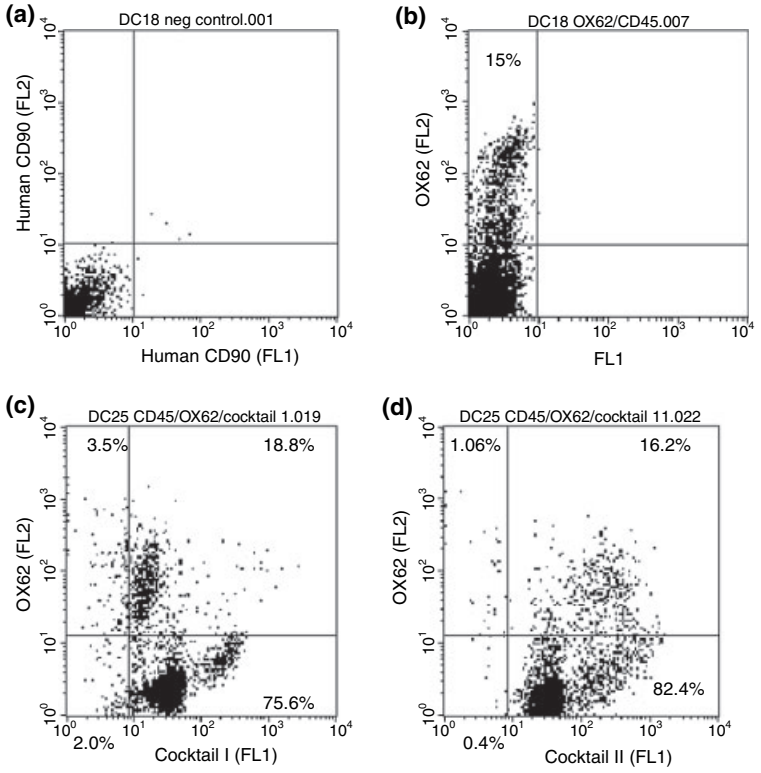

Figure 4 Flow cytometry analysis of OX62 binding to blood leucocytes. Blood leucocytes from a normal DA rat were analysed, using three colour flow cytometry on R4 gated cells (please refer to Fig. 1b). (a) Control antibodies, (b) analysis with the OX62 antibody only, (c) analysis with OX62 in FL2 and cocktail of fluorescein labelled antibodies in FL1 (CD3, CD45RA, CD161, ED1, TCR gamma/delta), (d) as in (c), except that fluorescein-labelled MRC OX6 antibody to MHC class II antigens is included in the cocktail.

dendritic cells were measured in six rats treated with four ALS injections, three rats treated with two ALS injections, and five normal or saline treated rats. Although there was a distinct trend to lower numbers of putative dendritic cells in ALS-treated rats, the numbers in the controls were too variable to enable definitive comparisons with the ALS-treated groups. The fact that these cells did not form a discrete group on flow cytometry (Fig. 4c) probably contributed to this variability.

Immunohistological studies

Rat heart contains two distinct types of tissue leucocytes, the interstitial dendritic cell and the tissue macrophage. It has previously been reported that whole body irradiation has no effect on the number of tissue macrophages, but results in the total loss of interstitial dendritic cells within 3–4 days [15,18]. However, the hearts of ALS treated rats have a normal complement of MHC class II positive interstitial dendritic cells (Fig. 5b). Tissue macrophages, stained with the ED2 antibody, were unaffected by ALS treatment (data not shown).

The fate of blood leucocytes exposed to ALS *in vitro* and *in vivo*

Figure 6a shows the scatter parameters for blood leucocytes in normal rat blood, with lymphocytes (R1), monocytes (R2) and granulocytes (R3) being readily distinguishable. These leucocyte populations can easily be detected in the blood of rats treated with intraperitoneal injections of ALS (Fig. 6b). However, if 1 ml of heat-inactivated ALS is added to 9 ml of fresh rat blood *in vitro,* and the blood analysed 1 h later, no leucocytes can be seen at all (Fig. 6c). This is not a consequence of the presence of residual rabbit complement, as mixing purified rat PBLs with the ALS *in vitro* does not lead to their lysis, as mentioned in a preceding section. The picture seen in Fig. 6c is almost certainly a consequence of rat complement-dependent, ALS-mediated leucocyte lysis. Complete lysis of leucocytes, although it happens *in vitro* (Fig. 6c), clearly does not happen *in vivo* after the intraperitoneal administration of ALS (Fig. 6b).

Discussion

Xenogeneic anti-lymphocyte sera are serologically complex, as would be expected from the presence of many hundreds of immunogenic proteins in the immunizing inoculum. An early serological analysis of rabbit antisera to purified rat T cells demonstrated that approximately half the antibodies in the antiserum were directed against broadly distributed tissue antigens, and the other half against leucocyte-specific antigens. The bulk of the leucocyte-specific component was directed at a single molecule, called the leucocyte common

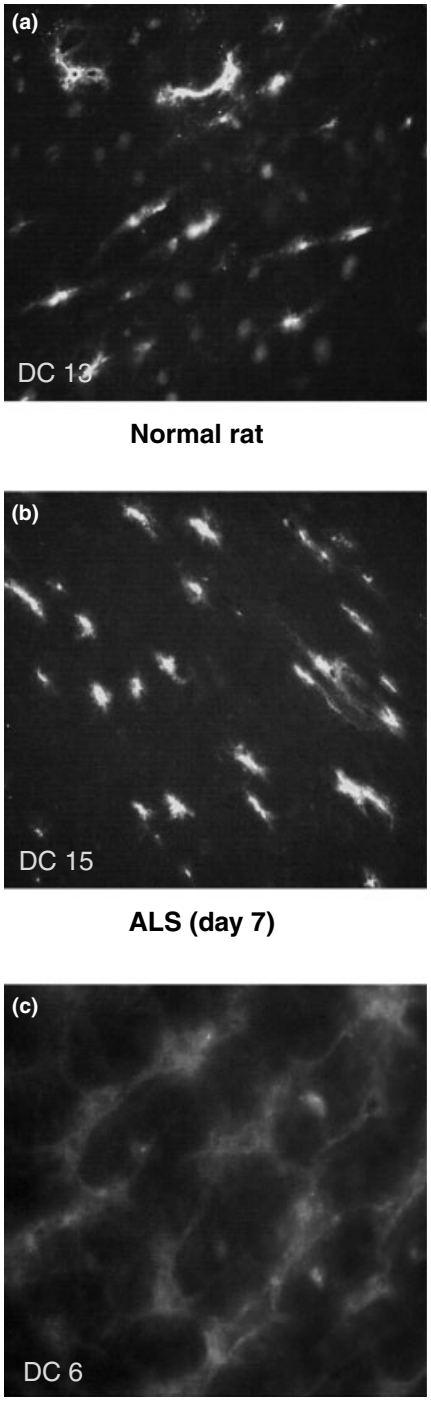

Figure 5 The effect of ALS on interstitial dendritic cells. Hearts from normal rats (a), rats treated with ALS on day 0, 1, 3 and 5 and removed at day 7 (b), and from rats given 1000 rads of whole body irradiation and removed at day 4 (c), were examined by immunohistology on frozen sections with the MRC OX6 antibody to MHC class II antigens. Control sections stained with the isotype control antibody were negative, similar to (c) (not shown).

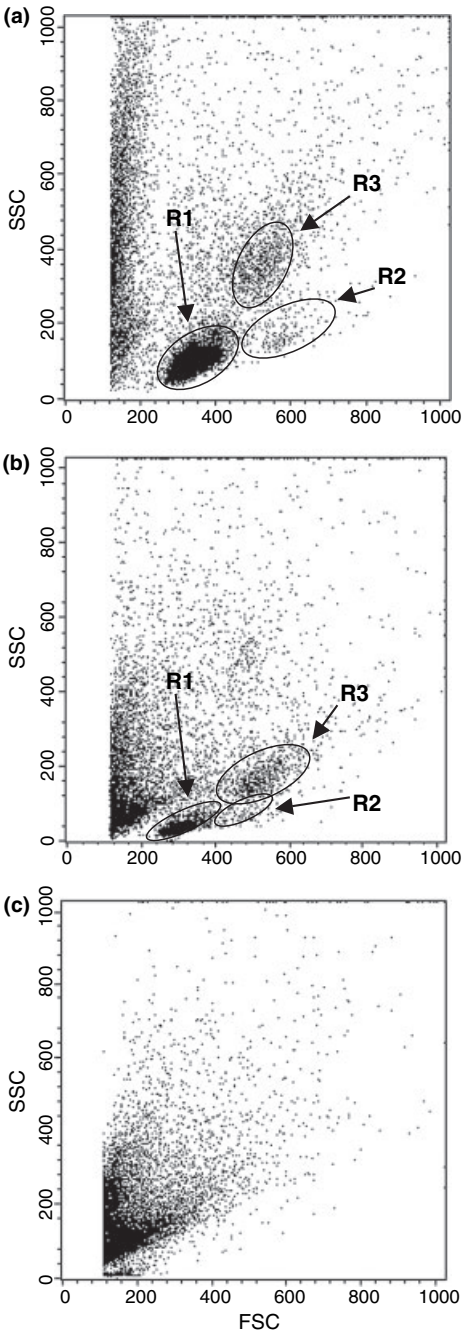

Figure 6 The fate of blood leucocytes. Blood from DA rats was analysed for forward (FSC) versus side scatter (SSC) profiles. The gated areas R1, R2, and R3 in (a) and (b) correspond to lymphocytes, monocytes and granulocytes, respectively. (a) Normal blood, (b) blood from a rat given ALS on days 0, 1, 3 and 5 and bled at day 7, (c) blood exposed to (heat-inactivated) ALS *in vitro*.

(LC) antigen [34], later designated CD45, which is present on all leucocytes. Although rabbit antibodies to pure rat CD45 were able to completely suppress the rejection of renal allografts in rats, large doses were required. Moreover,

specific removal of antibodies to CD45 from ALS did not diminish its immunosuppressive potency. It was clear that the potent immunosuppressive effects of ALS were mediated by a minor serological component [35]. That critical component has never been identified, which greatly complicates an assessment of possible mechanisms of action.

An early quantitative serological analysis of anti-human and anti-dog ALS [36] gave a tissue distribution pattern very similar to the anti rat ALS mentioned above, while the use of flow cytometry and immunohistochemistry showed that anti-human ALS reacts with plasma membrane as well as nuclear and cytoplasmic components of all the blood cells and solid organs evaluated [37]. When individual lymphocyte membrane antigens became better defined by the use of monoclonal antibodies, different batches of anti-human ALS produced in different species were analysed for the presence of these specificities [e.g. 38–41]. However, which (if any) of these specificities plays a role in the immunosuppressive properties of the ALS is unknown.

In our study, we have examined one batch of immunosuppressive rabbit anti rat thymocyte serum. Whether or not this batch of ALS is representative of ALS raised in other species against different human leucocyte populations using various protocols is uncertain. However, it raises the clinically important possibility that ALS might suppress innate immunity by depletion of monocytes and NK cells. ALS is usually monitored by measuring T cell depletion, so any associated depletion of NK cells, monocytes and blood dendritic cells will go unnoticed. These associated effects might vary with different batches of ALS or in different patients. It might be of clinical value to monitor these leucocytes of the innate immune system, to see if their depletion correlates with the suppression of rejection, or the development of opportunistic infections. Our study excludes a role for depletion of (either recipient or donor) interstitial dendritic cells as a mechanism of immunosuppression.

Lethal whole body irradiation results in the complete loss of interstitial dendritic cells within 3–4 days, presumably because of an interruption in the supply of bone-marrow derived blood precursors [15]. Although monocytes can be induced to differentiate into dendritic cells *in vitro,* the blood precursor for interstitial dendritic cells has not been positively identified. It is possible that relatively small numbers of monocytes are able to maintain a normal pool of interstitial dendritic cells, but the blood precursor of interstitial dendritic cells remains an open question.

It is interesting that the direct addition of heat-inactivated ALS to whole rat blood results in a rat complement-dependent lysis of *all* blood leucocytes. This is not seen when ALS is administered intraperitoneally *in vivo*. It might be the case that the broadly cross-reactive antibodies are absorbed out in the peritoneal cavity, and that only the leucocyte-specific component enters the blood circulation. This would diminish target antigen density on blood leucocytes, and might contribute to diminished complement-mediated ALS cytotoxicity. In clinical practice, pre-absorption of ALS with nonleucocyte tissues during its preparation might have the same effect.

Acknowledgement

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