

# Trephine biopsies are enriched for activated T/NK cells and cytotoxic T cells

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## Abstract

Although bone marrow aspiration is the most commonly obtained human marrow sample type, it yields liquid samples that contain a varying degree of blood contamination. Trephine biopsies, on the other hand, are solid marrow cores and are, therefore, much less likely to contain contaminating peripheral blood. In this study, we utilised a technique to extract viable cells from solid trephine biopsy specimens, by means of mechanical and enzymatic digestion, allowing cytometric comparison of cells in these biopsies and simultaneously obtained liquid aspirate samples. Having established that the digestion procedure itself was not causing any significant alteration in the immunophenotypic composition of the marrow samples, our data show that trephine biopsies were enriched for CD8<sup>+</sup> T cells, with concomitant decrease in the CD4<sup>+</sup> subset, compared to paired aspirates. Furthermore, T cells, NK cells and T cells expressing NK cell receptor (NKR) molecules were all significantly more likely to express both early (CD69) and late (HLA-DR) markers of activation. Bone marrow aspirates do not, therefore, provide truly representative data on the phenotypic composition of bone marrow, and the effect of peripheral blood contamination in aspirates should be taken into account when comparisons are being made between the bone marrow and other human issues or, perhaps more so, between human and murine marrow.

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## 1. Introduction

Bone marrow in the C57BL/6 mouse strain contains approximately six times the number of T cells expressing the NK cell receptor molecule NK1.1 (NK1.1<sup>+</sup> T cells) than are found in the peripheral blood of the same animals, accounting for up to 30% of the total marrow T cell pool [1]. However, no such enrichment for NK receptor (NKR)-expressing T-cells is seen in human bone marrow, either when examining the classical invariant V $\alpha$ 24J $\alpha$ 18 NKT cells [2] or when investigating expression of a broader range of marker molecules [3]. In the mouse, both conventional T cells and NKR<sup>+</sup> T

cells are known to play important roles in the recognition of and response to malignancies, with evidence that they are capable of acting as effectors themselves, causing rejection of malignant cells in an *in vivo* model system [4–6]. They also appear to function in a more indirect manner, coordinating the response by other immune cells, and NK cells in particular [7–9]. The proportions of NKR<sup>+/-</sup> T cells, and the functions attributed to them, differ markedly among tissues in the mouse [1,10]. There is evidence to suggest that the same is also true in humans, with some degree of tissue segregation indicated by NKR<sup>+</sup> T cells being highly enriched in the liver and gastrointestinal tract, compared to the periphery [11–13].

When sampling murine bone marrow, the whole marrow is harvested, the bone being either split open and the marrow removed, or the contents flushed out in their entirety with sterile medium. Clearly, this approach is not viable in hu-

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man studies and, instead, these generally focus on the use of bone marrow aspirates, drawing material from the marrow cavity with a syringe. However, the suction involved as marrow is drawn off, causes rupture of venous sinusoids running through the marrow, leading to varying degrees of 'contamination' of the marrow sample by peripheral blood. Such contamination can be largely avoided, and, perhaps, a more accurate representation of the immunological composition within the marrow obtained, by using an alternative sampling technique, the trephine biopsy, which yields a solid core of marrow, without blood contamination. Trephine biopsies are usually analysed as histological specimens, with only a few groups having processed them in order to extract cells from the bony matrix [14–16] and predominantly utilising the extracted cells in cytological preparations, regarding the processed trephine biopsy only as an alternative to be used in cases of "dry tap" aspiration [14–16].

As the sampling methodologies employed when conducting studies using human and murine bone marrow differ so markedly, comparisons of the immunophenotypic composition of marrow from the two species may not be wholly appropriate. We therefore set out to compare the phenotypic composition of human T/NK cells derived from solid trephine biopsy specimens with those in simultaneously obtained liquid aspirate samples, allowing evaluation of the use of the aspiration method in studies such as these.

## 2. Materials and methods

### 2.1. Patient samples

Bone marrow aspirates and trephine biopsies were taken from the posterior iliac crest of patients attending the haematology clinic in St. Vincent's University Hospital. Sample volume in excess to that necessary for routine diagnostic purposes was made available for this study and ethical approval was received from the St. Vincent's University Hospital Research and Ethics Committee. Seven matched bone marrow aspirate and trephine biopsy pairs were obtained from patients (four males, three females; median age 46.3 years, range 22.8–55.8) in remission following haematopoietic malignancies (two lymphomas, two myelomas and one each of acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL) and Hodgkin's disease). Aspirated samples were drawn from the marrow with a syringe through a 14-gauge needle, and were immediately transferred into RPMI 1640 medium containing 200 U/ml heparin. Trephine biopsies were obtained with a nine-gauge Jamshidi biopsy needle and then transferred into sterile sample collection tubes until processing.

### 2.2. Enzymatic digestion of trephine biopsies

Approximately one-third of each trephine biopsy (TB) was collected and washed by vortexing in 10 ml Hanks

balanced salts solution (HBSS; Gibco, Paisley, Scotland). The longer portion was used for routine diagnostic analysis in the Haematology Department. Cells contained within the biopsy fragments were released by enzymatic digestion, using a technique adapted from that used by Curry et al. [17] to extract viable mononuclear cells from human liver. Samples were cut into fragments of approximately 1 mm<sup>3</sup> in size with sterile scalpel blades and then incubated at 37 °C for 30 min, with constant rotation, in "digest medium" (HBSS containing 0.5 mg/ml type IV collagenase, 0.02 mg/ml DNase I (both Sigma-Aldrich, Dublin, Ireland), 2% foetal calf serum (FCS; Gibco, Paisley, Scotland) and 0.6% bovine serum albumin (BSA; Sigma-Aldrich, Dublin, Ireland)). After digestion, the solution was passed through a 30 µm filter mesh (Cadisch, London, England) to remove undissociated debris and centrifuged at 600 × g for 10 min. The resulting pellet was washed twice, firstly with "wash medium" (HBSS containing 0.02 mg/ml DNase, 2% FCS and 0.6% BSA) and secondly with HBSS alone, before resuspending in "complete" RPMI 1640 medium (supplemented with 10% heat-inactivated FCS, 25 mM HEPES, 2 mM L-glutamine, 50 mg/ml streptomycin and 50 U/ml penicillin, all obtained from Gibco, Paisley, Scotland).

### 2.3. Preparation of bone marrow mononuclear cells (BMMC)

Mononuclear cells were isolated from bone marrow aspirates by density gradient centrifugation. Briefly, aspirated marrow was diluted 1:2 with HBSS and overlaid onto Lymphoprep® (Nycomed, Norway) before centrifugation at 400 × g for 25 min, after which the accumulated mononuclear cell "buffy coat" was carefully removed. These cells were washed twice in HBSS, before resuspension in complete RPMI 1640 medium.

### 2.4. Effect of enzymatic digestion on cell surface marker expression

To ensure that any alterations in phenotype detected were not due to the enzymatic digestion, aliquots of bone marrow aspirate were centrifuged and the resulting cell pellets resuspended in "digest medium" and subjected to the same incubation (37 °C for 30 min) as the trephine fragments.

### 2.5. Flow cytometry

Cells from trephine biopsies and aspirates were stained for expression of a range of cell surface markers. Briefly, 5 µl of each fluorescently labelled monoclonal antibody (mAb), or relevant isotype control (all obtained from Becton Dickinson Immunocytometry Systems, Oxford, UK), were added to 100 µl cells in complete RPMI, mixed thoroughly and incubated at 4 °C for 30 min. Following incubation, the cells

Table 1

Median fluorescence intensity (MFI) of surface marker specific monoclonal antibodies before and after treatment with digest medium ( $n = 4$ )

Marker	Fluorochrome	Untreated MFI ( $\pm$ S.E.M.)	Treated MFI ( $\pm$ S.E.M.)	Untreated % positive ( $\pm$ S.E.M.)	Treated % positive ( $\pm$ S.E.M.)
CD3	PerCP	71.08 ( $\pm$ 13.30)	90.09 ( $\pm$ 10.95)	10.50 ( $\pm$ 0.86)	9.37 ( $\pm$ 0.92)
CD4	FITC	129.99 ( $\pm$ 10.27)	127.20 ( $\pm$ 8.16)	4.06 ( $\pm$ 0.89)	3.92 ( $\pm$ 1.09)
CD8	PE	1647.05 ( $\pm$ 87.38)	1445.12 ( $\pm$ 104.15)	7.08 ( $\pm$ 0.65)	6.57 ( $\pm$ 0.71)
CD45	FITC	782.02 ( $\pm$ 47.88)	782.02 ( $\pm$ 47.88)	16.10 ( $\pm$ 1.80)	14.20 ( $\pm$ 1.89)
CD45-RA	FITC	39.28 ( $\pm$ 3.21)	43.18 ( $\pm$ 2.71)	11.77 ( $\pm$ 2.82)	15.82 ( $\pm$ 3.15)
CD45-RO	PE	48.90 ( $\pm$ 5.78)	69.78 ( $\pm$ 14.54)	5.45 ( $\pm$ 0.76)	5.64 ( $\pm$ 0.71)
CD56	PE	42.40 ( $\pm$ 9.43)	52.96 ( $\pm$ 14.72)	6.48 ( $\pm$ 2.27)	6.24 ( $\pm$ 2.11)
CD57	FITC	124.88 ( $\pm$ 39.20)	210.63 ( $\pm$ 53.55)	5.19 ( $\pm$ 2.34)	3.82 ( $\pm$ 1.29)
CD69	FITC	42.60 ( $\pm$ 4.96)	43.91 ( $\pm$ 4.14)	3.41 ( $\pm$ 0.74)	2.66* ( $\pm$ 0.72)
CD161	FITC	22.88 ( $\pm$ 5.80)	17.84 ( $\pm$ 2.02)	1.39 ( $\pm$ 0.50)	2.07 ( $\pm$ 0.63)
HLA-DR	FITC	71.73 ( $\pm$ 17.47)	67.38 ( $\pm$ 19.06)	19.52 ( $\pm$ 1.70)	20.08 ( $\pm$ 3.37)
TCR $\alpha\beta$	FITC	89.04 ( $\pm$ 41.35)	51.99 ( $\pm$ 4.67)	8.17 ( $\pm$ 1.29)	9.71 ( $\pm$ 0.97)
TCR $\gamma\delta$	PE	51.71 ( $\pm$ 15.23)	41.57 ( $\pm$ 13.37)	2.14 ( $\pm$ 1.58)	0.94 ( $\pm$ 0.28)

Values represent average values for four independent experiments ( $\pm$ S.E.M.).\* Statistical significance ( $p < 0.05$ ), as determined by the Student's  $t$ -test for paired data.

were washed twice with PBS/BSA/azide (PBA) and centrifuged at  $600 \times g$  for 10 min. After the second wash, the cells were resuspended in 1% paraformaldehyde (PFA) fixative, prior to acquisition using a Becton Dickinson FAC-Scan flow cytometer and analysis using CellQuest software. Lymphoid cells were gated on forward/side scatter characteristics; CD45<sup>+</sup> or CD3<sup>+</sup> cells were then gated on FITC or PerCP fluorescence, respectively. Results are expressed as a percentage of the combined lymphoid and CD45<sup>+</sup>/CD3<sup>+</sup>

gates, to avoid skewing of results by granular cells within TB samples.

## 2.6. Statistical analysis

Statistical analysis of the relative proportions of different cellular populations investigated was performed using Student's  $t$ -test for paired data, with a  $p$ -value of less than or equal to 0.05 taken as statistically significant.

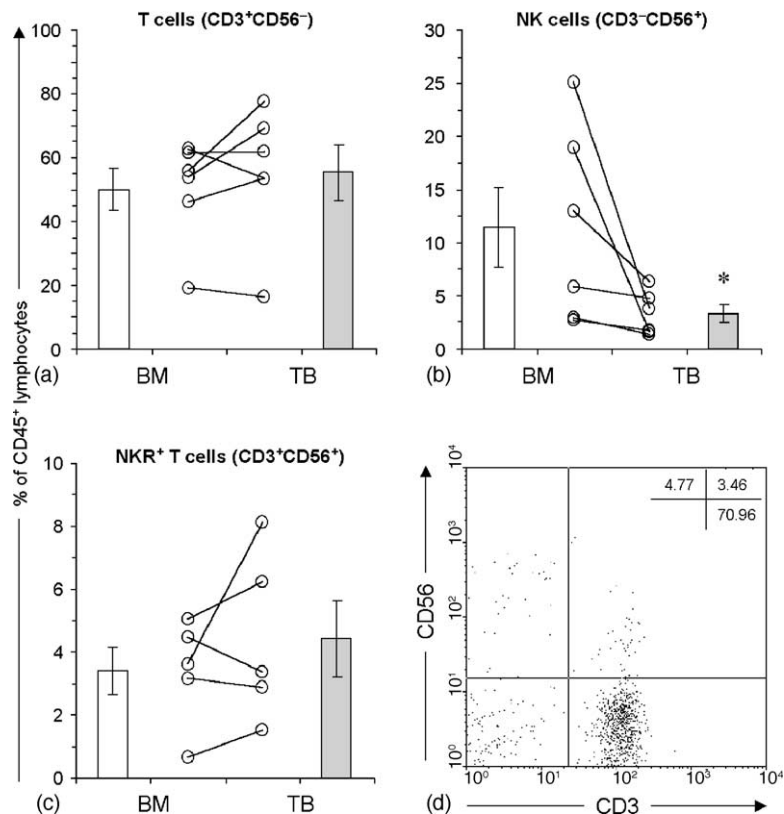


Fig. 1. Conventional T cell, NK cell and CD56<sup>+</sup> T cell contributions to the lymphoid pool in matched ( $n = 6$ ) bone marrow aspirate (BM) and trephine biopsy (TB) samples (a–c). Bars indicate average ( $\pm$ S.E.M.), while the dot plot shows representative data for one trephine biopsy sample (d). Numbers in the upper right hand corner indicate the relative proportions of CD3<sup>+</sup>CD56<sup>-</sup>, CD3<sup>-</sup>CD56<sup>+</sup> and CD3<sup>+</sup>CD56<sup>+</sup> cells.

### 3. Results

#### 3.1. Effect of enzymatic digestion on cell surface marker expression

In order to assess the effect of enzymatic digestion on the expression of cell surface markers, median fluorescence intensities (MFI) and percentage positivity for the markers examined in aliquots of treated or untreated marrow aspirate mononuclear cells were determined, and are summarised in Table 1. None of the molecules under investigation showed statistically significant differences in MFI following enzymatic digestion, while only one molecule, CD69, was detected on a significantly different proportion of treated cells, accounting for 2.66% of total cells after treatment, compared to 3.41% without ( $p < 0.05$ ).

#### 3.2. T/NK lymphoid cells

Having established that surface marker expression was essentially unaltered by the digestion process, we next compared the phenotypically determined T and NK cell populations in matched bone marrow aspirates and digested trephine biopsy samples. As Fig. 1 shows, NK cells ( $CD3^-CD56^+$ )

represent a significantly smaller proportion of lymphoid cells in trephine biopsies than in aspirated marrow samples (12.52% of  $CD45^+$  lymphoid cells in aspirates, compared to 2.52% trephine biopsies;  $p < 0.05$ ). Conventional T cells ( $CD3^+CD56^-$ ) and  $CD56^+$  T cells ( $CD3^+CD56^+$ ) were present at similar levels in trephine biopsies and aspirates. Likewise, no significant difference was seen between the levels of  $\alpha\beta$  or  $\gamma\delta$  TCR usage among T cells in the two sample types. There were, however, significantly fewer  $CD4^+$  T cells in trephine biopsies, with a corresponding increase in CD8 expression, compared to aspirated samples ( $p < 0.04$ , Student's *t*-test for paired data) (Fig. 2). The T cell populations expressing both CD4 and CD8 (double positives, DP) or neither molecule (double negatives, DN) were present at similar levels in both solid trephine biopsy specimens and matched aspirates (data not shown).

#### 3.3. $NKR^+$ T cells in trephine biopsies

With differential representation of the conventional CD4/CD8 T cell subsets in solid marrow trephine biopsies compared to liquid aspirates, it was next investigated whether the more unusual,  $NKR$ -expressing T cell subsets were comparable in these samples. As Fig. 3 shows, there was no signif-

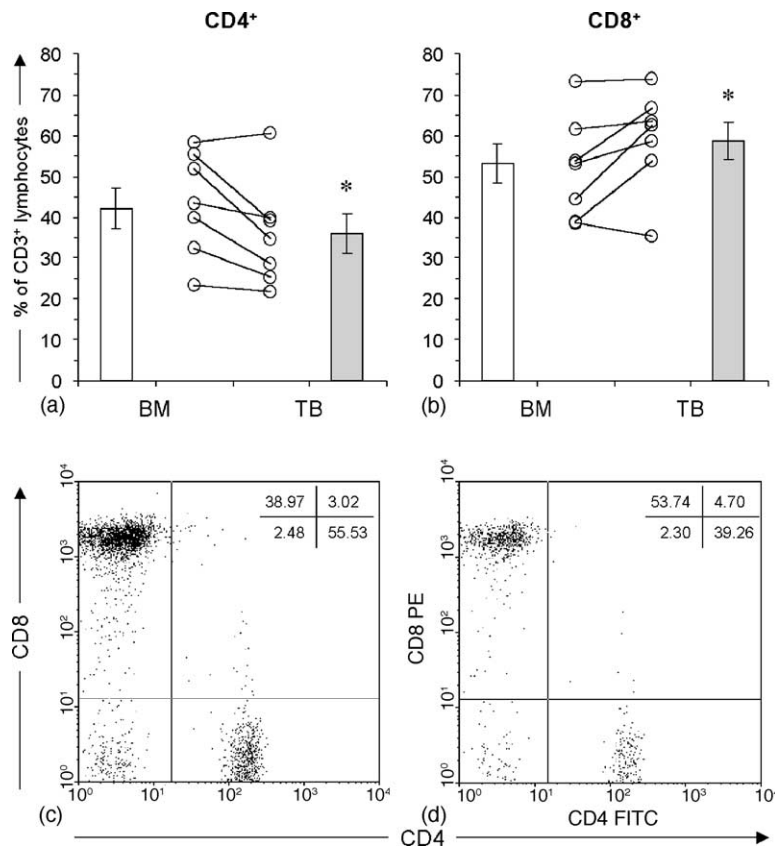


Fig. 2. Co-receptor expression by T cells in matched ( $n = 7$ ) bone marrow aspirates (BM) and trephine biopsy (TB) samples. The bars indicate average proportion of CD4 (a) or CD8 (b) positive T cells in each sample type ( $\pm$ S.E.M.). The dot plots show representative data for one matched pair of bone marrow aspirate (c) and trephine biopsy (d). Numbers in the upper right hand corner of each plot indicate the relative proportion of T cells accounted for in each of the four quadrants.

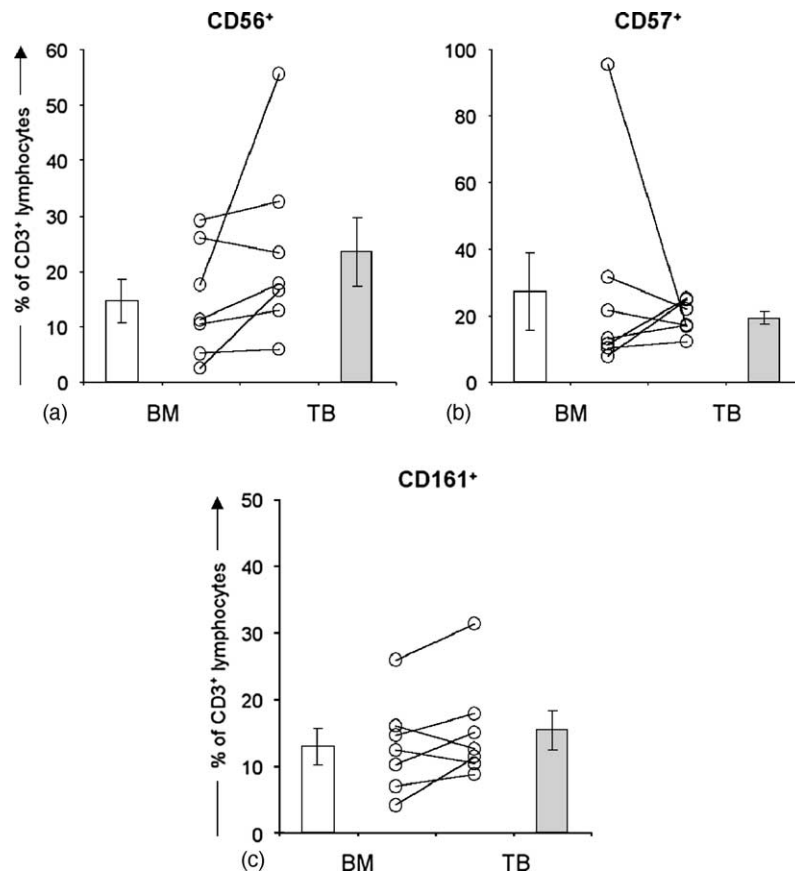


Fig. 3. NK cell surface marker molecule expression by T cells in matched ( $n=7$ ) bone marrow aspirate (BM) and trephine biopsy (TB) samples. The filled bars indicate average proportion of T cells expressing CD56 (a), CD57 (b) or CD161 (c), while the error bars indicate S.E.M.

icant difference in the average proportions of T cells expressing CD56 (a), CD57 (b) or CD161 (c). Thus, although NK cells ( $CD3^-CD56^+$ ) are significantly less common among trephine biopsy lymphoid cells (Fig. 1), T cells expressing the NK cell receptor molecules here which are likely to be important in tumour surveillance [4,5,18] are not diminished.

#### 3.4. Activation marker expression

Activation status, as determined by expression of early (CD69) and late (HLA-DR) activation markers by cells in bone marrow aspirates and paired trephine biopsies was next examined. Significantly more T cells expressed both activation markers in cells isolated from trephine biopsies than in aspirated samples. Indeed, average CD69 expression by trephine biopsy T cells was more than double than that seen in aspirates (50.64% of the trephine T cell pool, compared to 21.01% in aspirates,  $p < 0.0001$ ). Whilst not as pronounced, there was also a significantly larger proportion of HLA-DR positive T cells in trephines (48.14% of T cells) than in aspirates (30.44%,  $p < 0.0003$ ) (Fig. 4). This heightened level of activation is reflected not only in the overall T cell population, but also in the NKR positive and negative T cell subsets. As Table 2 shows, both CD69 and HLA-DR are significantly more likely to be expressed

by either conventional T cells ( $CD3^+CD56^-$ ) or NKR<sup>+</sup> T cells ( $CD3^+CD56^+$ ) in trephine biopsies than by the equivalent populations in aspirated samples. NK cells, too, showed greater expression of HLA-DR in trephine biopsies than in paired aspirates (54.88% of TB  $CD3^-CD56^+$  cells, compared to 16.32% in BM;  $p < 0.04$ ). Together, these data indicate that solid marrow, without the contamination from blood that occurs during the aspiration procedure, is enriched for activated cells that include both conventional T cells ( $CD56^-$ ) and NKR<sup>+</sup> cells (both NKR<sup>+</sup> T cells and NK cells).

#### 3.5. Effector/memory status

Elevated expression of activation markers by trephine biopsy T cells would suggest that they are receiving some form of stimulus in situ, therefore the effector/memory status of the T cells isolated from these biopsies was determined. As Fig. 5 shows, significantly fewer T cells in trephine biopsies than in paired aspirate samples expressed the CD45RA molecule alone (25.76% of trephine T cells, compared to 34.18% in aspirates,  $p < 0.03$ ). This is not accompanied by a corresponding increase in the CD45RO single positive population ( $RA^-RO^+$ ). However, there were significantly more

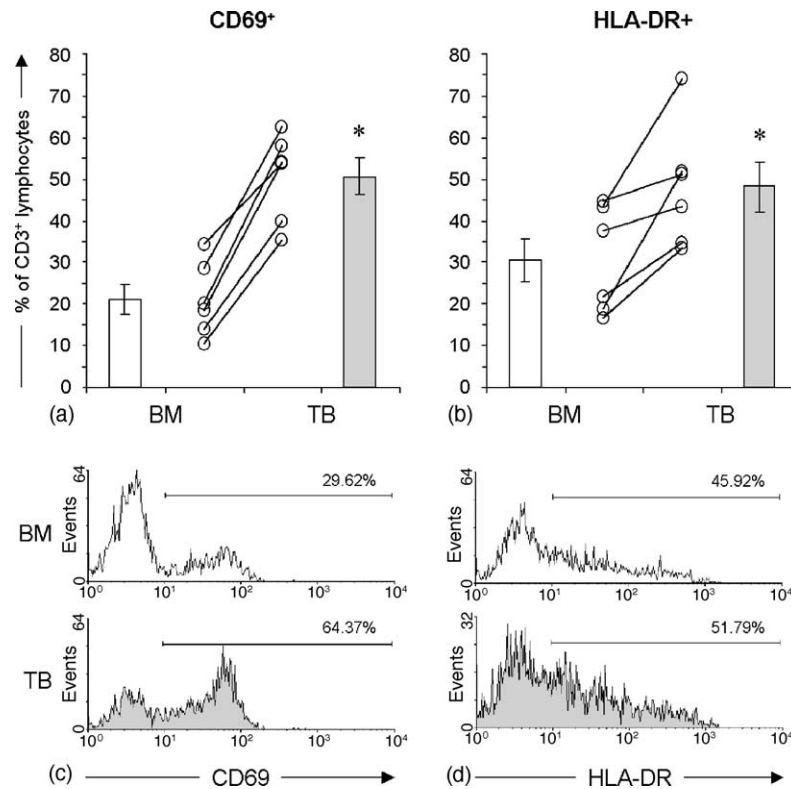


Fig. 4. Early (CD69) and late (HLA-DR) activation marker expression by T cells in matched ( $n=6$ ) bone marrow aspirate (BM) and trephine biopsy (TB) samples. The filled bars (a and b) indicate average proportion of T cells expressing each marker and the error bars indicate S.E.M. Histograms (c and d) show representative data for one pair of matched marrow aspirate (upper panels) and trephine biopsy (lower panels). Numbers indicate proportions of CD69 or HLA-DR expressing T cells.

cells expressing both molecules ( $RA^+RO^+$ ) among the T cells isolated from trephine biopsies (18.49%) than there were in aspirates (8.26% of T cells,  $p < 0.007$ ), suggesting the presence of recently activated cells. Thus, the solid marrow accessed by the trephine biopsy technique may contain not only a diminished pool of naïve T cells, but also a pool of cells in transition from a naïve to a memory phenotype. This tallies well with heightened activation already observed in T cells isolated from this tissue.

#### 4. Discussion

Although aspiration is the most common biopsy technique used in the clinical investigation of bone marrow, the rupture

of venous sinusoids in the marrow during the aspiration procedure leads to variable dilution of the marrow by peripheral blood. Trephine biopsies, on the other hand, involve the removal of a solid core of marrow, allowing for the minimum of contamination with blood and may more accurately represent the immune composition within the marrow. However, they represent a more severe procedure for the patient to undergo and are usually carried out in addition to prior aspiration [19]. However, in murine studies, marrow is generally obtained after sacrifice of the animals; the long bones are split and the whole marrow removed. Thus, trephine biopsy yields a marrow sample that may more closely resemble that used for murine experiments. Few groups have examined trephine biopsies by means other than histology [14–16], and doing so was primarily regarded as an option in the case of dry-tap as-

Table 2  
Activation marker expression by  $NKR^{+/-}$  T cell subsets and NK cells in paired ( $n=7$ ) bone marrow (BM) and trephine biopsy (TB) samples

Activation marker	Cell subset	BM% positive	TB% positive	$p$ -Value
CD69	$CD3^+CD56^-$	20.89 ( $\pm 3.77$ )	48.96 ( $\pm 4.60$ )	<0.001
	$CD3^+CD56^+$	25.12 ( $\pm 4.12$ )	65.37 ( $\pm 6.50$ )	<0.001
	$CD3^-CD56^+$	15.41 ( $\pm 4.91$ )	21.96 ( $\pm 6.18$ )	ns
HLA-DR	$CD3^+CD56^-$	30.36 ( $\pm 5.16$ )	46.29 ( $\pm 6.37$ )	<0.02
	$CD3^+CD56^+$	28.11 ( $\pm 6.74$ )	59.25 ( $\pm 8.38$ )	<0.002
	$CD3^-CD56^+$	16.32 ( $\pm 6.30$ )	54.88 ( $\pm 11.84$ )	<0.04

Values indicate the average percentage ( $\pm$ S.E.M.) of each cell subset expressing the activation markers CD69 or HLA-DR.  $p$ -Values were generated using Student's  $t$ -test for paired data. ns: not significant.

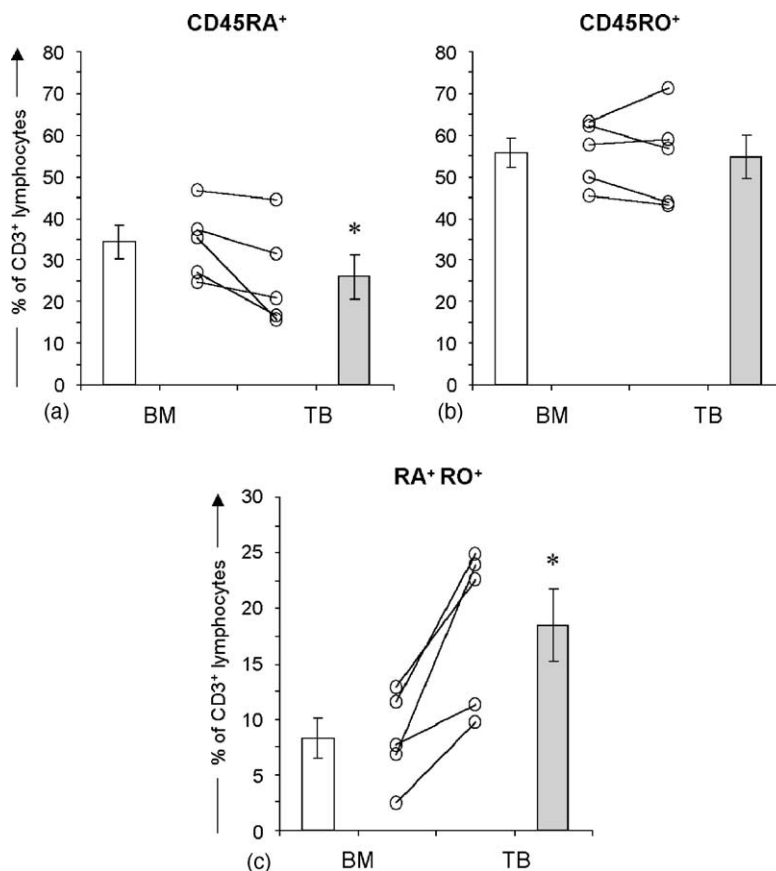


Fig. 5. Naïve/memory marker expression by T cells in matched bone marrow aspirate (BM) and trephine biopsy (TB) samples ( $n = 5$ ). The filled bars indicate average proportion of T cells expressing CD45-RA, CD45-RO or both molecules, and the error bars indicate S.E.M.

piration, i.e. where no readily stainable liquid sample could be obtained. In the present study, a technique for isolating viable immune cells from trephine biopsy cores by physical and enzymatic digestion, and subsequent staining and analysis by flow cytometry, was developed, based upon an earlier study [17], used to obtain mononuclear cells from liver biopsies.

Of initial interest was whether the digestion procedure itself would alter the phenotype of the cells. To test this, matched trephine biopsy and aspirate samples were subjected to the same processes, prior to flow cytometric analysis, such that any effect on the proportion or degree of positivity could be measured. Our data show that, in all but one case, neither the median fluorescence intensity, which can be taken as correlating directly with the number of target molecules on a cell surface [20], nor the proportions of marker-positive cells was significantly different for the panel of markers used in the phenotypic investigation. The single exception to this was a slight, yet significant, decrease in the overall proportion of cells staining positive for CD69. However, this also served to confirm that the digestion process was not causing activation of the cells. In this study, we used a combination of CD45<sup>+</sup> or CD3<sup>+</sup> and lymphoid gates to quantify lymphoid populations, thus preventing skewing of the results by granular cells in trephine biopsy samples, which had not been subjected to

density gradient centrifugation. However, the possible loss of cell subsets during centrifugation of aspirates [21] could not be ruled out by this approach. Staining of matched marrow aspirates before and after mononuclear cell isolation to address this possibility showed no significant alterations in the phenotypic composition of isolated cells (data not shown).

Phenotypic analysis showed that NKR positive and negative T cells showed no reduction among trephine biopsy lymphoid cells, compared to matched aspirates. Likewise, the CD56<sup>+</sup>, CD57<sup>+</sup> and CD161<sup>+</sup> T cell subsets represented similar proportions of the total T cell pool in both aspirates and trephines, suggesting that the aspirate sample is equally as good as trephine biopsy for examining these T cell populations. This highlights that there is, indeed, a significant difference between the levels of NKR<sup>+</sup> T cells in human and murine bone marrow. There were, however, significant differences between aspirate and trephine in almost every other cell population examined. NK cells (CD3<sup>-</sup>CD56<sup>+</sup>) were significantly less numerous in trephine biopsies than in their matched aspirates. CD8<sup>+</sup> T cells were more common, and CD4<sup>+</sup> T cells correspondingly less common, in trephines, enhancing the already enriched CD8<sup>+</sup> T cell pool detected in the marrow. These data clearly indicate how contamination from the bloodstream can affect the apparent prevalence of cell populations identified on the basis of surface pheno-

type. In this case, the CD4:CD8 ratio appears to be increased through blood admixture—CD4<sup>+</sup> cells predominate in the periphery, yet CD8<sup>+</sup> cells appear to be more abundant in the marrow.

Perhaps the most striking difference is the enriched pool of activated cells detected in trephine biopsies. More than double the number of trephine T cells express CD69 than do those in matched aspirates, and there is also a significant increase in HLA-DR positivity. Within the CD56<sup>+</sup> T cell subset, this increase is even more dramatic, and both CD69 and HLA-DR are expressed by more than twice as many NKR<sup>+</sup> T cells in trephine biopsies as in aspirates. One can exclude the possibility that these data are due to the digestion procedure stimulating the trephine biopsy cells, as the initial investigation has shown HLA-DR positivity to be unaffected by the digestion process and that CD69 experiences a drop in its expression levels. The result, therefore, appears to be genuine, and not an artefact of the digestion procedure. Although they are already high in aspirates, the trephine biopsy activation marker expression levels more closely resemble those seen in other organs possessing regional immune systems [22–24], and in murine marrow [1], perhaps indicating an immune composition in the bone marrow tailored to region-specific protective requirements. Analysis of the solid marrow samples also shows that the T cells contained in the marrow, without contamination and dilution with blood, are enriched for CD45RA<sup>+</sup>RO<sup>+</sup> cells. The nature of the changes in CD45 isoform expression suggests that enrichment of the RA<sup>+</sup>RO<sup>+</sup> population indicates an ongoing shift from naïve to effector/memory phenotype [25], possibly indicating that the cells are receiving some form of stimulus in situ.

In summary, our data suggest that trephine biopsies offer a more accurate representation of the phenotypic composition of human bone marrow than do the more commonly used aspirates. Trepines, however, are unlikely to replace aspirates in future studies, as the procedure is much more invasive, is much less frequently used and yields considerably fewer cells for analysis than aspirates. On the whole, the differences in phenotype detected by using trephine biopsies are exaggerations of those already detected when comparing aspirates to peripheral blood [3]. While this supports the notion that the ‘true’ composition of bone marrow is diluted by peripheral blood contamination during the aspiration procedure, it also means that aspirates probably allow the majority of phenotypic changes occurring in the marrow to be detected. However, while their frequency and higher cell yield is of obvious benefit to both the clinician and researcher alike, analysis of bone marrow aspirates may not provide truly representative data on the phenotypic composition of bone marrow, and the dilution effect from peripheral blood contamination during the aspiration procedure should certainly be taken into account when the data is analysed and when comparisons are being made between the bone marrow and other human organs. It is also worth noting the stark contrast between the NKR<sup>+</sup> T cell enrichment seen in murine bone marrow [1]

and the absence of such enrichment in humans, even when whole, solid marrow is examined.

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