Clin Exp Immunol 1994; 97:120–125

# Altered expression of CD11/CD18 on the peripheral blood phagocytes of patients with tuberculosis

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*(Accepted for publication 11 April 1994)*


## SUMMARY

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is characterized by granulomatous lesions made up of epithelioid cells, giant cells and mononuclear leucocytes. Cell–cell adhesion is important in granuloma formation and in the leucocyte migration which accompanies it. We have recently shown increased expression of the adhesion molecules CD11/CD18 (LeuCAMs, $\beta_2$ integrins) on peripheral blood leucocytes from patients with sarcoidosis (Shakoor & Hamblin, 1992). Here we have studied the expression of CD11/CD18 and CD29 (VLA $\beta_1$ integrin) on the peripheral blood leucocytes of 10 TB patients by flow cytometry. The density (expressed as mean fluorescence intensity) of CD11b on monocytes and polymorphs was increased ($P < 0.005$), as was CD11c ($P < 0.005$) and CD18 ($P < 0.05$) on polymorphs. CD11a expression was significantly reduced on polymorphs ($P < 0.05$). No differences were found in the expression of CD29, the percentages of cells expressing any molecule and, in contrast to sarcoidosis, the density of any molecule on lymphocytes. Although the cytokine tumour necrosis factor (TNF) has been implicated in the process of up-regulation, an ELISA for TNF failed to detect significant levels in plasma. The results suggest increased peripheral phagocyte CD11/CD18 expression is a feature of TB, which may contribute to the pathological processes involved.


**Keywords** CD11/CD18 tuberculosis leucocytes tumour necrosis factor integrins

## INTRODUCTION

Infection with *Mycobacterium tuberculosis* leads to the activation of macrophages and lymphocytes and to the formation of granulomata [1,2]. Accumulation of monocytes, lymphocytes and polymorphs occurs at the sites of infection following cell adhesion to endothelial cells and migration from blood vessels into tissues [3,4]. Cell–cell adhesion is essential for interaction between leucocytes, for granuloma formation and for the migration of leucocytes through vessel walls. These adhesive processes are mediated by a variety of adhesion proteins divided into families [5]. Amongst these, the $\beta_1$ and $\beta_2$ integrin adhesion molecules play important roles in the extravasation of leucocytes. Thus, MoAbs against the $\beta_1$ and $\beta_2$ integrins block cell–cell and cell–matrix interactions [5–7], and cells from patients with leucocyte adhesion molecule deficiency (LAD), which fail to express any cell-surface $\beta_2$ integrins, have decreased phagocyte adhesion related functions [8].

The role of adhesion molecules may be moderated by both quantitative and qualitative changes in their expression [9]. Increased levels of expression of the $\beta_2$ integrins CD11/CD18 have been associated with increased cell adhesion and migration both *in vivo* and *in vitro* [9,10], and may be brought about by certain cytokines such as tumour necrosis factor-alpha (TNF-$\alpha$), TNF-$\beta$, IL-1, interferon gamma (IFN-$\gamma$), IL-2, and IL-4 [11,12]. In addition, cell activation by cytokines results in cells shedding adhesion molecules, which may then be detected in a soluble form in serum. Increased levels of the adhesion proteins intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin have been reported in the serum of patients with tuberculosis [13,14].

We have previously shown that sarcoid patients have increased CD11/CD18 expression on their peripheral blood leucocytes [15] and have increased soluble E-selectin in their circulation [16]. Sarcoidosis and tuberculosis (TB) both represent chronic granulomatous conditions with local tissue invasion by leucocytes. Evidence exists that mycobacterial DNA is found in patients with sarcoidosis, suggesting a similar infectious etiology to TB [17].

The principal aim of this study was to quantify the expression of adhesion proteins on the peripheral blood leucocyte populations of patients with TB using flow cytometry, and thus seek similarities or differences with our findings in sarcoidosis. The molecules studied represented the $\beta_2$ integrins CD11a/CD18, CD11b/CD18 and CD11c/CD18, and the $\beta_1$ integrin

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VLA-$\beta$ chain (CD29), the expression of which is increased on the synovial fluid T cells of patients with rheumatoid arthritis (RA) [18]. Since abnormal proportions of peripheral lymphocyte T cell subpopulations are found in sarcoidosis [15,19] and have been reported in TB [20], we have investigated lymphocyte proportions in the TB patients described here. Our finding of an increase in phagocyte integrin expression led us to measure levels of serum TNF-$\alpha$.

## PATIENTS AND METHODS

*Study population*

Patients were selected on the basis of either a positive sputum test for acid-fast bacilli and chest x-ray shadowing, or a lymph node biopsy which was positive for *Myco. tuberculosis*. The length of treatment, smoking habits, alcohol consumption, weight status, general health of the patients and any other medication were recorded (Table 1). The patients consisted of three Caucasians, two Chinese, three Afro-Caribbeans and two Asians, and had a mean age of 39 years (range 22–67 years) (Table 1). Patients were compared with healthy age-, sex- and race-matched controls. Since lymphocyte abnormalities have been reported as being greatest in untreated patients [20], we compared the results for patients with a recent diagnosis within 4 weeks or less ($n = 6$), and therefore short periods of treatment, with those whose diagnosis had been 12 weeks before venesection ($n = 4$).

*Preparation of leucocytes*

The technique of rapid fix lysis [21] was used to prepare peripheral blood leucocytes for flow cytometry. Blood was kept at 37°C and processed within 1 h of being taken. Briefly, 1·0 ml of heparinized (20 U/ml; Leo Laboratories, Princes Risborough, UK) blood was immediately fixed by the addition of 1·0 ml PBS (Dulbecco's A; Oxoid, Basingstoke, UK) containing 0·4% formaldehyde (BDH, Poole, UK) and incubated at 37°C for 4 min in a universal container (Bibby-Sterilin, Stone, UK). Then, 20 ml of pre-warmed (37°C) 0·83% ammonium chloride (BDH) in Tris (0·01 mol/*l*) (Fisons, Loughborough, UK) lysing solution were added to the fixed blood and incubated at 37°C for 4 min to allow erythrocyte lysis. The cells were centrifuged at 160 *g* for 5 min and the resultant leucocyte-rich pellet was washed three times by resuspending in 10 ml PBS and re-centrifuging. Finally, the cells were resuspended in 0·5 ml ice-cold RPMI (Gibco, Paisley, UK) containing 5% fetal calf serum (FCS) and kept on ice until use.

*Monoclonal antibodies*

All the mouse MoAbs were IgG1. Leu-4 (CD3), Leu-16 (CD20), Leu-3a (CD4) and Leu-2a (CD8) (Becton Dickinson, Mountain View, CA), were used to give the proportions of T lymphocytes, B lymphocytes and CD4 and CD8 T cell subpopulations, respectively. CD45RO (UCHL1; Dakopatts, Glostrup, Denmark) and CD45RA (Leu-18; Becton Dickinson) delineated putative memory and naive T cell subsets. Class II MHC expression was examined using HLA-DR antibody (Becton Dickinson), and monocytes were identified by CD14 (Leu-M3; Becton Dickinson) expression. MHM24 (CD11a), KB90 (CD11c) (Dakopatts), CD11b (Biogenesis, Bournmouth, UK), LFA-1$\beta$ (CD18) and 4B4 (CD29) (Coulter, Hialeah, FL) identified the adhesion molecules. Antibodies were either PE-conjugated (HLA-DR, CD20, CD4 and CD8) or FITC-conjugated. Unlabelled cells and isotype controls (IgG1-FITC, IgG1-unconjugated (Becton Dickinson)) were run in parallel.

*Cell staining*

Staining involved the addition of 10 $\mu$l of the relevant MoAb to the wells of a flexible microtitre plate (Falcon, 3911). Previous studies had shown that 10 $\mu$l of all the antibodies saturated the target cells. Cell suspension (25 $\mu$l) was added to each well and mixed with the antibody by gentle tapping. The plate was then incubated over ice, in the dark, for 30 min. After incubation, three drops of 5% FCS/RPMI were added to each well and the plate centrifuged at 160 *g* for 5 min. The cells were washed twice with four drops of 5% FCS/RPMI, and re-centrifuged as above. Finally, after resuspending in one drop 5% FCS/

**Table 1.** Clinical details of patients with tuberculosis

| Patient | Age (years) | Ethnic group/sex* | Time since diagnosis | Site of lesion† | Treatment‡ | Weight status§ | Smoking habits¶ | Alcohol intake** |
|---|---|---|---|---|---|---|---|---|
| 1 | 54 | C/M | 4 days | L | P,A | D | — | > 50 |
| 2 | 22 | C/M | 0 days | L | Ni I | D | — | 5 |
| 3 | 59 | C/M | 12 weeks | L | Pr, R, Py | D | — | 5 |
| 4 | 33 | Ch/F | 16 weeks | L | R, I, Py, Pr | D | — | — |
| 5 | 25 | AC/F | 7 days | L | R, E | D | — | — |
| 6 | 38 | AC/M | 20 weeks | N | R, P, Py | N/C | — | 24 |
| 7 | 67 | A/F | 16 weeks | L | Pr, R | N/C | — | — |
| 8 | 24 | AC/M | 2 weeks | L | I, R, P | N/C | 25 | 50 |
| 9 | 35 | A/F | 4 weeks | L | R, Py | N/C | — | — |
| 10 | 33 | Ch/M | 3 weeks | N | R, I, Py, P | N/C | — | — |

* C, Caucasian; A, Asian; AC, Afro-Caribbean; Ch, Chinese.
† L, Lung; N, lymph node.
‡ P, Pyrazinamide; A, amitriptyline; Pr, prednisolone; R, rifampicin; Py, pyridoxine; E, ethambutol; I, isoniazid.
§ N/C, No change; D, down.
¶ Cigarettes/day.
** Units/week.

RPMI, the cells were transferred to FACScan tubes (Falcon, 2054), containing 300 $\mu$l of 1% paraformaldehyde (BDH) in PBS and left overnight at 4°C, in the dark, before flow cytometry.

*Flow cytometry*

Flow cytometry was performed using a FACScan (Becton Dickinson) and LYSYS II software (Becton Dickinson), as previously described [21]. Ten thousand events were collected for each sample. Cells were gated on the basis of forward scatter and side scatter into lymphocytes, monocytes and granulocytes. The ability of the gates to segregate cells was checked using CD14 and CD3 antibodies to determine the purity of the monocyte and lymphocyte gates, respectively; less than 3% cells in the monocyte gate expressed CD3, and less than 0·4%

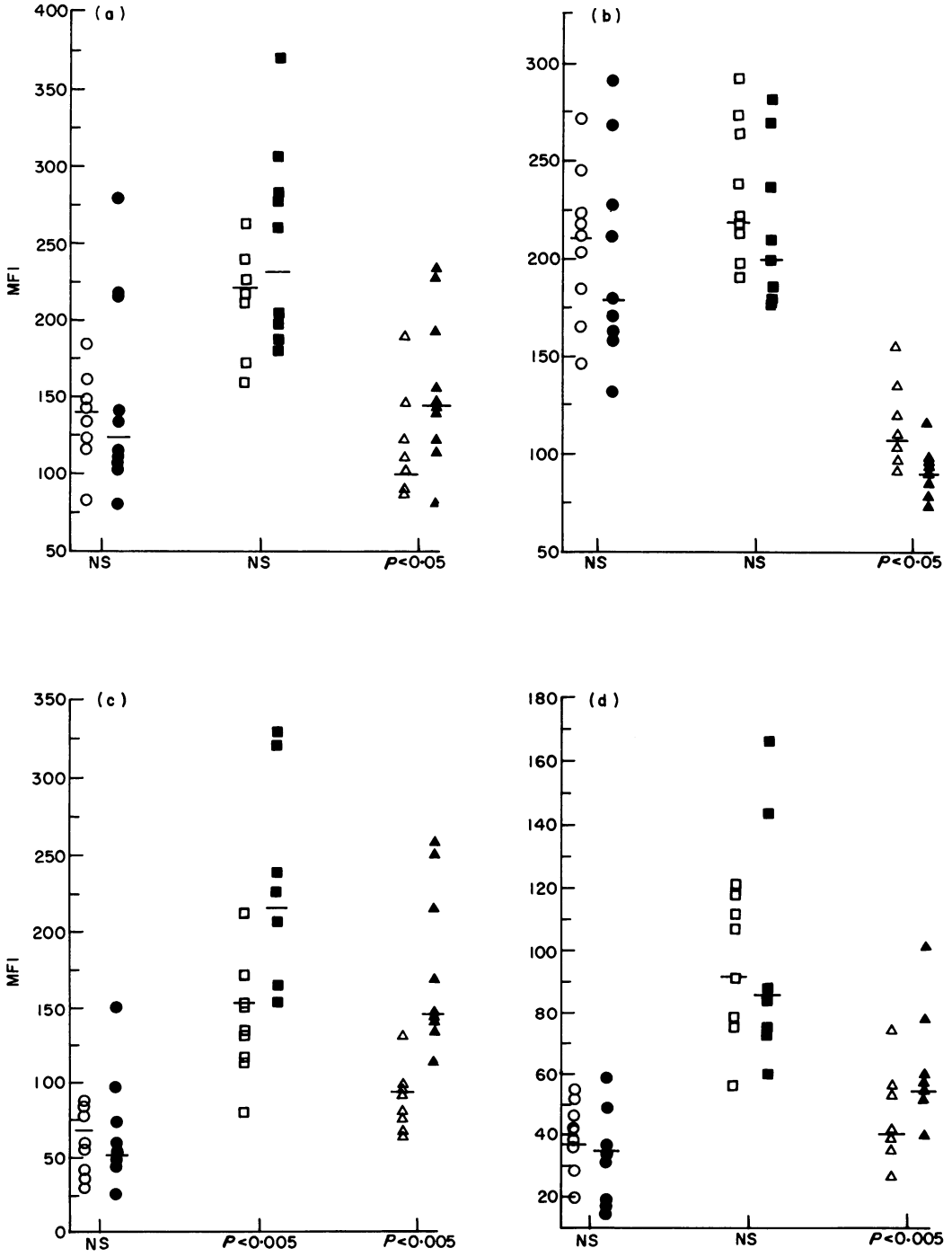


**Fig. 1.** Expression of (a) CD18, (b) CD11a, (c) CD11b, and (d) CD11c on lymphocytes (circles), monocytes (squares) and polymorphs (triangles) from patients (closed symbols) and controls (open symbols). Horizontal bars represent median values, as calculated by the Wilcoxon rank test. NS, Not significant.

cells in the lymphocyte gate expressed CD14. Alignment of the detectors and compensation remained constant throughout the study. Controls were used to give a measure of non-specific binding, against which results were expressed as percentages of positively staining cells and as a mean fluorescence intensity (MFI), in arbitrary units transformed to a linear scale from the $\log_{10}$ channel number of mean fluorescence, for a particular cell marker.

*TNF assay*

Plasma samples were obtained by centrifugation of heparinized blood, and were stored at −20°C. Total TNF-$\alpha$ levels in all samples were measured using a commercial Enzyme Amplified Sensitivity Immunoassay (Medgenix, Milton Keynes, UK). The minimum limit of detection for the assay was 3 pg/ml TNF, where the normal range was 3–20 pg/ml. Absorbance was read at 450 nm.

*Statistical analysis*

Comparisons between the MFI of patients' and controls' leucocyte adhesion molecule expression on lymphocytes, monocytes and polymorphs were made using the Wilcoxon rank test: variation is expressed as the 95% confidence interval (CI). Percentages of cells staining for a particular marker were expressed as means ± s.d., with significance calculated with Student's *t*-test. Significance was accepted at the 5% level.

## RESULTS

*CD11/CD18 expression*

Levels of CD11b on patients' monocytes were increased compared with controls ($P < 0.005$), although there was no significant difference in the level of expression of HLA-DR; median MFI patients 388 (95% CI 257–519) *versus* median MFI controls 352 (95% CI 268–436). Expression of CD11b ($P < 0.005$), CD11c ($P < 0.005$) and CD18 ($P < 0.05$) on patients' polymorphs was increased, whilst that of CD11a was decreased (Fig. 1). Lymphocyte CD11/CD18 expression was not significantly different between patients and controls (Fig. 1). No correlation was seen between the levels of expression and any aspect of disease.

In accordance with our previous studies [15] the percentage of cells expressing CD11/CD18 was no different between patients and controls (data not shown).

*CD29 expression*

Comparison of patients with controls showed that no differences were seen in either the level of expression or in the percentages of any cell types expressing CD29. In accordance with published data [22] CD29 was expressed mostly on monocytes (median MFI patients 120 (95% CI 95–150) *versus* median MFI controls 141 (95% CI 121–161)), and at a low level on lymphocytes (MFI patients 44 (CI 35–52) *versus* MFI controls 49 (CI 33–68)). CD29 was not expressed on polymorphs.

*Lymphocyte populations*

No significant differences between patients and controls were found in the percentages of T cells, activated T cells ($CD3^+$/$HLA\text{-}DR^+$ cells), B cells, CD4 or CD8 T cell populations, and CD4 or CD8 T cells expressing the naive and memory T cell markers (Table 2). Division of patients into those with a diagnosis within less than 4 weeks or more than 12 weeks made no difference to these findings.

**Table 2.** Lymphocyte populations and subpopulations

| Marker | Patients | Control |
|---|---|---|
| CD3 | 71 (8·5) | 72 (5·2) |
| CD3/HLA-DR | 9 (5) | 11 (8·1) |
| CD20 | 11 (4·8) | 14 (8·1) |
| CD4 | 45 (8·5) | 42 (8·2) |
| CD8 | 33 (11·2) | 38 (12·1) |
| CD45RO/CD4 | 27 (7·6) | 38 (6·6) |
| CD45RO/CD8 | 18 (9·9) | 13 (6·3) |
| CD45RA/CD4 | 18 (12) | 17 (5·2) |
| CD45RA/CD8 | 22 (9·1) | 25 (7·1) |

Results shown as mean% (± s.d.).

*TNF-$\alpha$*

No difference was seen in the levels of total plasma TNF-$\alpha$ between patients and controls. All but two values were within the normal range of 3–20 pg/ml. The median values (95% CI) of the patient and control group were 10·34 pg/ml (8·7–12·0) and 9·32 pg/ml (6·5–12·1), respectively.

## DISCUSSION

In this study we were able to show increased CD11/CD18 expression on both monocytes and polymorphs in a group of patients with proven TB, but with a variable time since diagnosis and with various regimes of treatment (Table 1). This pattern was similar to that seen in sarcoidosis [15], with the exception that CD11a on polymorphs was decreased. Although we were unable to show increased HLA-DR on monocytes, as was found in sarcoidosis [15], the findings are consistent with phagocyte activation in the two diseases, either by cytokines or microbial products.

Monocyte activation as assessed by various functional and phenotypic markers has been reported previously in TB [23–25]. Thus blood monocytes have been reported to show increased adherence to plastic [23], to show increased expression of the Fc receptor for IgG [24], and to secrete increased amounts of IL-1 [25]. Recently it has been shown that monocytes have increased expression and occupancy of cell surface receptors for TNF-$\alpha$ [26]. TNF-$\alpha$ has been implicated in the up-regulation of CD11/CD18 [12,13], and is known to be released by macrophages activated by mycobacterial antigens [27,28]. However, we failed to find increased TNF-$\alpha$ in the patients' serum using an immunoassay which would detect TNF-$\alpha$ both bound and unbound to the soluble TNF-$\alpha$ receptor, and thus total circulating TNF-$\alpha$. This supports the view that TNF-$\alpha$, which might cause CD11/CD18 up-regulation, is cell bound [26] and may exert its effect when cells pass through local production sites such as the lung. We did not assay for other circulating cytokines which have been shown to increase the expression of phagocyte CD11/CD18 *in vitro* [12,13], and which have been implicated in mycobacterial disease [28], leaving open the possibility that another circulating cytokine may be elevated and responsible for the increased expression on peripheral cells.

The role played by polymorphs in the chronic stages of mycobacterial infection is not clear [29], although their accumulation at sites of infection has been reported [3,4,29]. This process may be facilitated by the increased expression of adhesion proteins seen here. The decreased expression of CD11a may be due to specific down-regulation by transcriptional repression, as has been reported for the cellular oncogene c-myc [30], or may be due to the influx of CD11a-negative neutrophils under the influence of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [31]. Such mechanisms would imply the differential regulation of one component of the CD11/CD18 cell surface family in TB.

In this study patients did not show increased expression of CD11/CD18 molecules on circulating lymphocytes as has been reported in sarcoidosis [15]. In that disease the alterations in CD11/CD18 expression were accompanied by reductions in the numbers of circulating T cells, predominantly the CD4 T cells [15]. Here the proportions of lymphocytes and their subpopulations were no different between patients and controls. In addition, in this small group we could demonstrate no effect of length of treatment. Ainslie *et al.* [20] also found normal proportions of $CD3^+$ T cells in TB patients, but found a decreased CD4:CD8 ratio in peripheral blood, with a corresponding increase in bronchoalveolar lavage fluid. Antituberculous treatment was shown to increase the CD4:CD8 ratio in peripheral blood over a period of 3 months. Severity of disease or the effects of treatment may account for our failure to confirm the findings of Ainslie *et al.* [20]. Although the relationship of abnormal lymphocyte CD11/CD18 expression in sarcoidosis to other lymphocyte abnormalities remains to be determined, our failure to find increased CD11/CD18 on TB lymphocytes may be because the patients' T cells were present in normal proportions.

We did not see differences in the expression of CD29, the VLA $\beta$ chain, on patients' cells. VCAM-1, which is the endothelial ligand for VLA-4, is found in increased levels in the circulation of patients with TB [13], and up-regulation of CD29 has been demonstrated on synovial fluid but not peripheral blood T cells in RA [18], suggesting this immunomodulatory event occurs locally. Thus it may be of interest to examine CD29 on T cells from granulomata.

We have now studied a number of different groups of patients with varying diseases for expression of leucocyte CD11/CD18. Increased expression has been seen in sarcoidosis [15] and in HIV disease [32], but not in RA [33], inflammatory bowel disease [34], chronic vasculitis (Ahmad & Hamblin, unpublished data), and multiple sclerosis (Vora & Hamblin, unpublished data). It is tempting to speculate that chronic infections, as opposed to other immunopathological lesions, may produce the immunological conditions which favour this up-regulation. Such findings would lend weight to the idea that sarcoidosis is an infection, evidence for which has been provided by some [17] although refuted by others [35].

Whether such elevated CD11/CD18 expression has any functional consequences which contribute to the disease process can only be speculated. Whilst quantitative changes in expression have been associated with increased adhesion *in vitro* [5,9,10], it is evident that levels of increased adhesion of, for example, monocytes to endothelial cells do not correlate with levels of increased adhesion protein expression [36], and that several CD11/CD18-mediated functions can occur without an increase in the level of expression on the cell surface [37]. However, increased expression may predispose cells to increased adhesion when they are exposed to a further inflammatory stimulus [15].

In conclusion, we have shown altered CD11/CD18 expression on phagocytes from TB patients. Increased expression may arise from activation by cytokines or microbial products, and may facilitate cell adherence and accumulation of cells at sites of infection.

## ACKNOWLEDGMENTS

We thank the Smith and Nephew Foundation for sponsoring R.J.Y. Thank are due also to Dr N. Bateman, Dr C. Twort and Dr V. Gant of St Thomas' Hospital; Dr P. Rees, Dr M. Cochrane and Dr H. Milburn of Guy's Hospital; Dr R. Shaw of St Mary's Hospital and Dr D. W. Williams of Ealing General Hospital for their help in finding clinical samples.

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