JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 3 (1992) 151 - 153

Thermochemical studies of surface-induced activation in human granulocytes *in vitro*

J. IKOMI-KUMM, L. LJUNGGREN*, M. MONTI
*Department of Medicine and * Department of Clinical Chemistry, University Hospital of Lund, S-22185 Lund, Sweden, * Protein Laboratory, University of Copenhagen, Denmark*

The technique of microcalorimetry has been extensively applied in studies of metabolic processes in human blood cells in suspension. In contrast to other types of blood cells, human granulocytes and other phagocytic cells are activated by contact with the stainless steel vessel in which the sample under study is enclosed. The activation is characterized by the production of increased quantities of heat energy with variable time-dependent profiles. The present study demonstrates that lining the stainless steel vessels with fluoroethylene–polypropylene will completely eliminate the contact activation of granulocytes. It is shown that this modified procedure enhances the quality of microcalorimetry as a technique for the measurement of granulocyte metabolism and function under physiological conditions *in vitro*.

1. Introduction

Direct calorimetry is a thermodynamic technique suitable for the study of energy transformations in biochemical systems such as plasma, whole blood, cell suspensions and tissue biopsies. The heat observed in a cell suspension by calorimetry is a summation of the heat generated in anabolic and catabolic reactions. During a calorimetric measurement the cells under study are in intimate contact with a chosen type of vessel of defined composition, structure and volume. Stainless steel (SS) has generally been accepted as a suitable material for these kinds of vessel when performing calorimetric studies on biological materials in general. The choice of SS was justified by qualities like high heat conductivity, tensile strength and apparent biocompatibility with the human blood cells. Using stainless steel vessels and standardized procedures, the metabolism of the human blood cells has earlier been evaluated with good precision from their rates of heat production, P (units $J\,s^{-1}$ per cell) [1]. Data obtained using erythrocytes, platelets and lymphocytes from humans have been applied in clinical investigations to confirm and define the abnormal metabolism of blood cells in anaemia, thyroid disease and non-Hodgkin lymphoma [2–4].

However, a similar application has been unsuccessful with respect to granulocytes. The P values for these cells in plasma suspension are unreproducible in contrast to those for suspensions of lymphocytes, erythrocytes and platelets which are lower and constant [5]. There seems to be a need for blood-compatible materials which do not trigger endogenous cellular processes for studies of granulocytes. The ideal material should have such properties as chemical and thermal stability and be free from processing aids or additives. Perfluorocarbon polymers represent a unique class of such material from which we have selected fluorinated ethylene–propylene (FEP). In earlier works Teflon-coated ampoules were used but their effect on cell metabolism remained unevaluated [6].

2. Experimental procedure

Venous blood was taken from healthy, male, non-fasting blood donors at the blood bank of the University Hospital, Lund. 20 ml blood was collected from each donor into Vacutainer tubes containing heparin at 13 U ml^{-1}. Granulocytes were isolated from the blood by centrifugation on a density gradient in a one-step procedure using Mono-Poly Resolving Medium (Flow Laboratories, USA). On this gradient granulocytes and mononuclear cells are separated in two distinct bands with retained functional properties [7–9]. Contaminating erythrocytes were removed by hypotonic lysis at 0 °C using 0.3% sodium chloride for 20 s, then made isotonic with 4.5% sodium chloride and centrifuged. Washed granulocytes were resuspended in cell-free autologous plasma buffered with 50 mM tris to pH 7.4

In a set of experiments to determine the effect of SS on the rate of heat production in granulocytes, either 0.1 or 0.2 ml of the suspension containing 10^6 cells were enclosed in sealed 1 ml cylindrical SS ampoules. In another set of experiments using similar experimental conditions the granulocyte suspensions prepared from blood obtained from another group of healthy donors were enclosed in SS ampoules, the internal surfaces of which were lined with 13 μm thin sheet of FEP (Dupont, USA). This was accomplished by fitting a thin foil of FEP with a cylindrical piston into the ampoule in the form of a cup. Prewetting of the ampoule with 0.1 ml of physiological saline was used to keep the FEP foil in intimate contact with the wall and bottom of the ampoule. This arrangement prevented contact between the SS surface and the cell suspension under study.

0957–4530

The basal rate of heat production, P (in the absence of external stimulus) was recorded for 2 h under static conditions at 37 °C. The ampoules were then reopened outside the calorimeter and 15 mg heat-sterilized zymosan (Sigma, USA) in 50 µl saline was added to the granulocyte suspension with gentle mixing. The rate of heat production was recorded for another period of 2 h. Calorimetry was performed using a prototype of the Thermal Activity Monitor (Thermo Metric AB, Sweden). A functional scheme of this heat conduction microcalorimeter is shown in Fig. 1.

3. Results

When performing calorimetric measurments on plasma suspensions of granulocytes in SS ampoules under static conditions, we consistently recorded initially high rates of heat production, P, which decreased rapidly and reached low values after about 2 h as illustrated in Fig. 2 (curve a). Complete elimination of this activation profile was achieved when FEP-lined ampoules were used as illustrated by the power–time curve b in Fig. 2. The P value determined using FEP-lined ampoules, buffered plasma and granulocytes from a group of nine blood donors was 1.44 ± 0.10 pW per cell (mean ± SE) at pH 7.4. For this group the coefficient of variation in P values obtained for double calorimetric determinations on cell suspensions from each donor was 5%. For granulocytes from the other group of 10 donors and comparable experimental conditions but using unlined SS ampoules, the mean P value was 2.21 ± 0.20 pW per cell. The difference between the two groups is statistically significant ($p < 0.01$).

The distribution of the P values for the two groups is shown in Fig. 3. Stimulation of the granulocytes with zymosan gave very high initial P values which decreased with time. Both the initial P value and the value after 2 h were higher for samples in contact with FEP than with SS. The activation index (P_I) for the granulocyte response to zymosan particles was calculated from the relationship

$$P_I = \frac{P_a - P}{P} \times 100$$

where P and P_a are the rates of heat production in the granulocyte suspension with and without zymosan, respectively. P_I was (467 ± 55)% for granulocytes in the FEP-lined ampoules and (99 ± 15)% for the samples in the SS ampoules. The difference between the two activation indices is highly significant ($p < 0.005$).

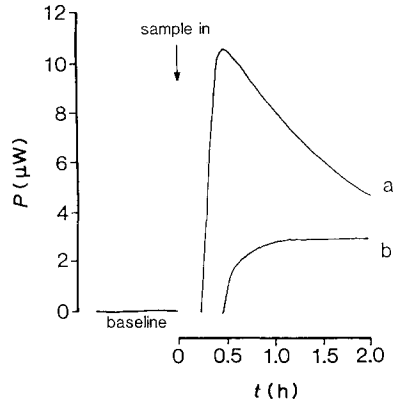

Figure 2 Power–time curves (calorimetric profiles) for granulocyte suspensions in (a) SS ampoules and (b) FEP-lined ampoules.

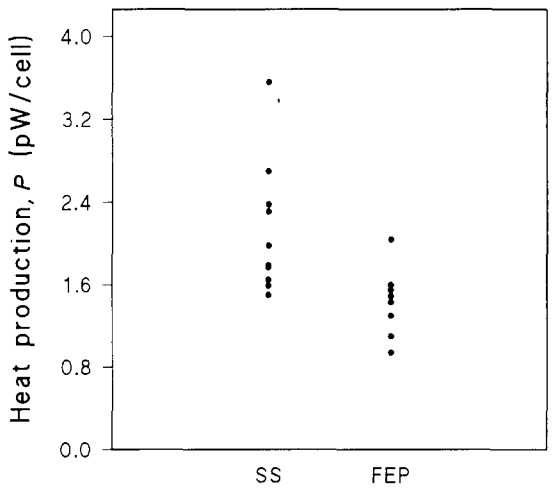

Figure 3 Illustration of the basal heat production from two series of measurements of granulocytes in contact with the different materials FEP and SS.

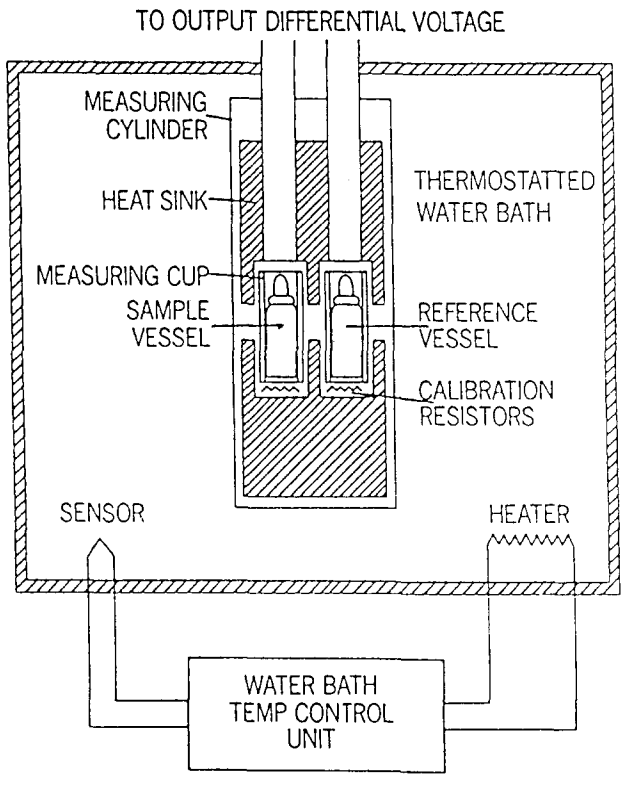

Figure 1 Functional diagram of the Thermal Activity Monitor.

4. Discussion

In the non-activated state, granulocytes show low activity of the hexose monophosphate shunt and glycolytic pathway [10]. When granulocytes interact with membrane-perturbing agents, both soluble and particulate, both pathways are stimulated to generate energy-rich adenosine triphosphate (ATP) and molecules with bactericidal properties. This metabolic

response has previously been demonstrated by microcalorimetry as marked and variable increases in heat production for granulocytes responding to various stimuli such as heat-killed staphylococcal protein A-IgG [11] and antigen–antibody complexes [12].

The observed amplified metabolic signal from resting granulocytes in contact with the SS surface of the ampoule is a summation of heat energy generated by metabolic reactions, heterogeneous in character. This initial surface-related activation has a depressive effect on subsequent granulocyte phagocytic response to particles. The effect is undesirable as it introduces variable and systematic errors which reduce the specificity of the calorimetric technique as applied to the study of granulocyte metabolism and function. The finding that this unwanted activation process could be eliminated by FEP is of interest.

Our results suggest that the beneficial effect of FEP may be explained by the elimination of cellular interaction with the SS surface. The cellular interaction may be related to the effect of plasma protein deposition, which is known to occur on artificial surfaces [13, 14]. The surface characteristics of SS may increase chemotactic activity of granulocytes by activation of the complement system or induce conformational changes of IgG in plasma. That an FEP analogue PTFE (polytetrafluoroethylene) has been found to be inert to the complement system as well as to granulocytes [15] supports our findings.

The decrease in immune response of granulocytes to zymosan caused by the previous activation on the SS surface may be associated with membrane alterations and/or energy depletion. Cell adhesion to the ampoule surface and phagocytosis of foreign bodies on SS are energy-consuming processes which could induce initially high rates of oxygen consumption followed by hypoxia and reduction of ATP production.

As internal surface imperfections have been shown to be present in SS materials [16], FEP seems to be a more suitable standard reference material, not only for calorimetric vessels when performing metabolic studies on cells. It is evident that granulocytes are highly reactive cells with a high capacity for oxidative metabolism. These qualities may thus be exploited for their use as sensors for surface impurities *in vitro*. The results strongly indicate that the use of FEP will enhance the quality and specificity of calorimetric studies of granulocyte metabolism and function. There is a good correlation between calorimetry and chemiluminescence to measure granulocyte activation. However, only calorimetry provides quantitative information on the consumed chemical energy involved in the activation process [17].

Acknowledgements

The technical assistance of Mrs B. Persson is gratefully acknowledged. The work has been supported by grants from the Nordic Fund for Technology and Industrial Development and from Påhlsson's Foundation, Malmö, Sweden.

References

1. M. MONTI, in "Thermal and Energetic Studies of Cellular Biological Systems", edited by A. M. James (Wright, Bristol, UK, 1987) p. 131.
2. M. MONTI and I. WADSÖ· *Scand. J. Clin. Lab. Invest.* **32** (1973) 47.
3. M. MONTI, J. IKOMI-KUMM and S. VALDEMARSSON, *Thermochim. Acta.* **172** (1990) 157.
4. M. MONTI, L. BRANDT, J. IKOMI-KUMM, H. OLSSON and I. WADSÖ, *Scand. J. Hematol.* **27** (1981) 305.
5. U. BANDMANN, M. MONTI and I. WADSÖ, *Scand J. Clin. Lab. Invest.* **35** (1975) 121.
6. I. WADSÖ, *LKB Instrum. J.* **21** (1974) 18.
7. A. FERRANTE and Y. H. THONG, *J. Immunol. Meth.* **36** (1980) 109.
8. L. P. BIGNOLD and A. FERRANTE, *ibid.* **96** (1987) 29.
9. L. P. BIGNOLD, *Cell Biol. Int. Rep.* **11** (1987) 19.
10. C. EFTIMIADI and G. RIALDI, *Cell Biophys.* **4** (1982) 231.
11. *Idem., Microbiologica* **8** (1985) 225.
12. R. FÄLDT, J. ANKERST, M. MONTI and I. WADSÖ, *Immunology* **46** (1982) 189.
13. C. F. MANDENIUS and L. LJUNGGREN, *Biomaterials* **12** (1991) 369.
14. D. F. WILLIAMS, I. N. ASKILL and R. J. SMITH, *Biomed. Mater. Res.* **19** (1985) 313.
15. A. REMES, PhD thesis, University of Liverpool (1990).
16. L. CARLSSON, *Kemisk Tidskrift* **4** (1990) 26.
17. C. EFTIMIADI and G. RIALDI, *Thermochim. Acta* **85** (1985) 489.

Received 16 October 1990
and accepted 4 February 1991