Acta Paediatrica Hungarica 29 (1–2), pp. 203–207 (1988–89)

Gingival crevicular studies in patients with neutrophil dysfunction

MJ Kowolik, JA Raeburn

University of Leeds School of Dentistry and The Host Defence Laboratory, Human Genetics Unit, University of Edinburgh, UK.

Neutrophil granulocytes constitute 90–100% of the leucocyte population of the human gingival crevice where they respond to the chemotactic challenge of plaque bacteria, as on other mucosal surfaces. They are crucial to homeostasis but also contribute with both protective and to a lesser extent, destructive response mechanisms to the pathogenesis of the periodontal diseases [1, 10, 16].

The importance of neutrophils to host defence is seen in the high morbidity and mortality still associated with deficiency states, particularly those of a quantitative nature. In cyclical neutropenia, for example, life-threatening bacteraemia and septicaemia may be caused by oral commensal bacteria, and early-onset, rapidly destructive periodontitis underline the vital role of the cell to normal health [5, 12]. Neutropenia is often a feature of leukaemic states and is one result of cytotoxic chemotherapy.

Purely functional disorders of neutrophils present a less serious threat to survival, but can predispose to infection. The most intensively studied condition is chronic granulomatous disease (CGD), in which the intrinsic defect is in the oxygen-dependent killing system. Several variants exist, but classically the disease is X-linked and thus identification of both the boys affected and the female carriers within a family is important [2]. Studies of gingival crevicular neutrophils (GCN) have confirmed the integrity of the oxygen-dependent system and demonstrated its high activity [8, 9, 10]. Being easily accessible, the cell should also provide a marker of migration and systemic function, and this paper describes investigations performed on crevicular cells from individual patients with both qualitative and quantitative disorders.

Materials and Methods

All methods used for the collection, counting and functional testing of both peripheral venous blood (PBN) and gingival crevicular neutrophils (GCN) have been described in detail previously [6, 8, 9, 10, 15].

Neutropenia and quantitation

1) In a 9 year-old boy with congenital neutropenia, GCN were collected and counted during a neutropenic phase for comparison with the PBN values. A nitro-blue tetrazolium (NBT) test was also performed on both groups of cells.

2) Cell counts were performed on 17 occasions in a 25 year-old female patient with acute myelomonocytic leukaemia, from the time of diagnosis to the time of her death, a period of three months.

3) A 15 year-old girl with acute monocytic leukaemia became febrile and septicaemic after a period of remission, and was shown to have severely depressed bone marrow function. According to a standard protocol, two successive granulocyte transfusions were given, using her 17 year-old HLA-typed brother as donor. Neutrophils were collected with a continuous flow cell separator, 2 units (1.9×10^{10} cells/unit) being transfused with a 17 hour interval between. For several days, no neutrophils, had been detectable in the peripheral blood and very few in crevicular washings. Cell counts were monitored prior to, during and after the 2 transfusions. An NBT test was performed on the GCN sample at 132 hours post-transfusion.

Functional Assays

A) A 53 year-old man with chronic myeloid leukaemia (CML) was studied over a 4 year period, from diagnosis until the terminal phase of his illness. Among tests carried out on both PBN and GCN were a cytochemical assay of neutrophil alkaline phosphatase (NAP), according to a standard technique [3], performed at the time of diagnosis, and a similar assay of myeloperoxidase (MPO) in the GCN [4], performed during and after the commencement of treatment.

B) Further to a previous study [11], investigations were carried out in a family known to be affected by CGD. Nitroblue tetrazolium tests were performed on PBN and GCN from a 6 year-old boy with the X-linked disease and also on cell from both parents.

Results

Although variable, GCN counts, using a standardised rinsing technique [16], are in the range $1-12\times10^4$/ml washings ($1-12\times10^7$/1). Normal PBN values were taken as $2.2-9.0\times10^9$/1.

Neutropenia and quantitation

1) In the 9 year-old boy, the neutropenic state was confirmed by a PBN value of 0.62×10^9/l, but the GCN count was normal at 1.7×10^7/1. The NBT test performed on both cell groups gave a normal result.

2) Figure 1 shows the PBN and GCN counts over 90 days for the patient with acute myelomonocytic leukaemia. The initially high PBN count was due to the large number of blast forms present. In general it can be seen that the GCN counts paralleled those of the PBN, with the former starting to decline first, in the terminal stage.

3. Figure 2 shows the kinetics of neutrophil passage into the crevicular fluid following granulocyte transfusion. Of importance was the observation that cells were easily collected from the exudate fluid some considerable time before being detectable by routine haematological screening of the peripheral blood samples. The GCN NBT test value at 132 hours was 43.1% positive cells (control value 42%), and compares to 11.6% during a phase of active disease.

Functional assays

A) The NAP scores for the patient with CML demonstrated a reflection of the expected low PBN value in the GCN, being 22 (120) and 62 (187) respectively. Control figures are in

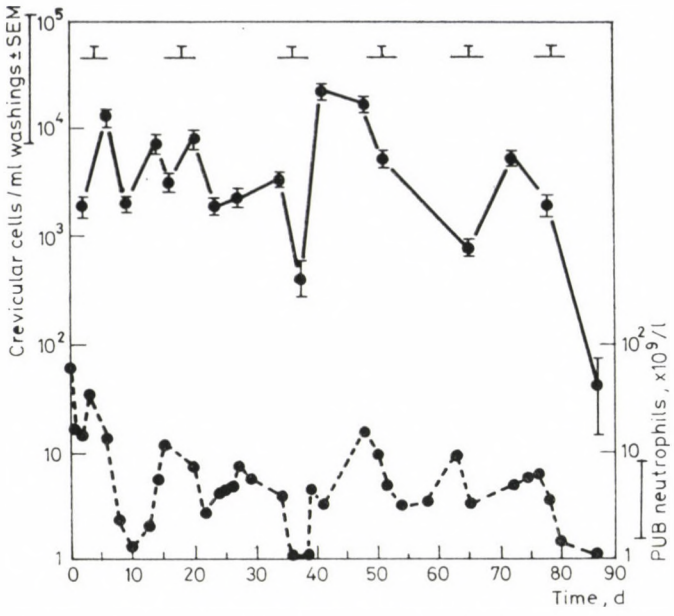

FIG. 1. Peripheral venous blood (— — — —) and gingival crevicular (————) neutrophil counts in a 25 year-old female patient with acute myelomonocytic leukaemia. The bars marked T represent periods of chemotherapy, and the error bars show the sem for 10 counts

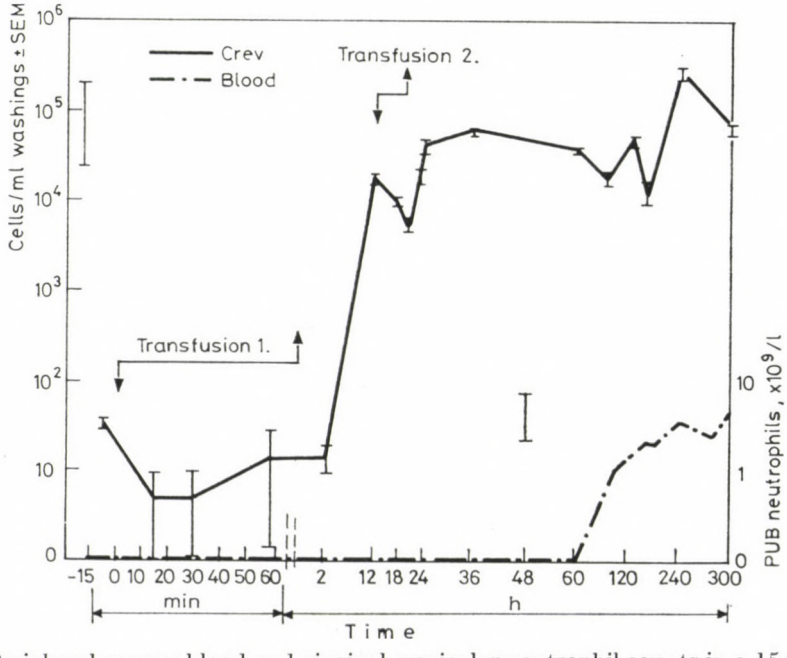

FIG. 2. Peripheral venous blood and gingival crevicular neutrophil counts in a 15 year-old female patient with acute monocytic leukaemia in response to 2 granulocyte transfusions. Normal values are shown by the vertical bars and the error bars show the sem values for 10 counts of the crevicular cells

brackets. The MPO scores were, before treatment, 67, 10 days after commencement, 84 and 45 days after commencement 185, i.e. at a normal level.

B) In the 6 year-old boy with X-linked CGD, neither PBN nor GCN responded in the unstimulated or stimulated NBT test. Cells from the father gave a normal response, while those from the mother a value 44% normal control.

Discussion

Neutrophils migrating into the gingival crevice are functional cells and have chemotactic ability similar to that of PBN [14]. Systemic deficiencies, whether in number or function, could be reflected in these cells, although selective migration of sub-populations of cells may occur [8, 9]. Indeed, in the neutropenic boy studied, the GCN count was normal. Together with the data for patient 3, where GCN appeared soon after transfusion, it would appear that the crevicular area is an important site of migration. Furthermore, the cell counts performed for patient 2, showed that GCN were always detectable even when PBN were not, as on days 10 and 35 – 40 (fig. 1). It is likely that, in the absence of an intravascular pool, transfused cells rapidly marginate and pass to the tissues. Thus it is probable that the PBN detected after 60 hours, and certainly after 120 hours, were of host origin, with recovery (temporary) of the bone marrow following.

In congenital neutropenia cell function is usually normal [5], and this was supported by the NBT test results for patient 1 and after treatment, for patient 3. The detection of functional alterations in GCN was also demonstrated in patient A, with CML, firstly by confirmation in GCN of the typically low NAP value, and then of the gradual improvement in cytochemical MPO score, with treatment. Myeloperoxidase may be a useful marker in leukaemias, with prognostic value [7, 13]. At no time were blast cells seen in the exudate fluid, and this is to be expected if only functional cells are able to migrate from vessels, via connective tissues onto mucosal surfaces.

Previous work had demonstrated the ability to detect CGD carrier status from GCN NBT test [11]. The results presented here, from another family, confirm this by illustrating the absolute lack of response in both PBN and GCN from a boy with CGD, and the partial response of GCN from his mother.

Neutrophils in the gingival crevice can be collected easily and atraumatically, so that monitoring of systemic functions by this method may be helpful in the severely ill patient or where few cells are available, as in the neutropenic states.

Acknowledgement

We are grateful to Dr. N. C. Allan for permitting us to study patients under his care (2,3 and A), and to Mr. R. Sutherland for his invaluable technical assistance.

References

1. Genco RJ, Slots J: Host responses in periodontol diseases. J Dent Res 63: 441, 1984
2. Hitzig WH, Seger RA: Chronic granulomatous disease, a heterogeneous syndrome. Human Genet. 64: 207, 1983
3. Kaplow LS: Cytochemistry of leukocyte alkaline phosphatase. Am. J. Clin Pathol 39: 439, 1963
4. Kaplow LS: Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 26: 215, 1965
5. Kay AB, White. AG, Barclay GR, Darg C, Raeburn JA, Uttley WS, McCrae WM, Innes EM: Leucocyte functions in a case of chronic benign neutropenia of infancy associated with circulating leucoagglutinins. Br J Haematol 32: 451, 1976
6. Kowolik MJ: Quantitative and cytochemical studies of human gingival crevicular neutrophils: myeloperoxidase activity and nitroblue tetrazolium reduction. PhD Thesis, University of Edinburgh, 1984
7. Kowolik MJ: Sensitivity of gingival crevicular neutrophil myeloperoxidase to chemotherapy. In: The Borderland Between Caries and Periodontal Disease eds. Lehner, T., Cimasoni, G. Medecine et Hygiene, Geneva 1986, p. 319
8. Kowolik MJ: The Oxygen-dependent Microbicidal System in Human Gingival Crevicular Neutrophils. In: Advances in the Biosciences, Vol. 66, eds. Mauri, C., Rizzo, C., Ricevuti, G. Pergamon Journals Ltd., Oxford 1987, p. 175
9. Kowolik MJ, Grant M: Myeloperoxidase activity in human gingival crevicular neutrophils. Archs Oral Biol 28: 293, 1983
10. Kowolik MJ. Raeburn JA: Functional integrity of gingival crevicular polymorphonuclear leucocytes as demonstrated by NBT reduction. J Periodont Res 15: 483, 1980
11. Kowolik MJ, Raeburn JA: NBT reduction by exudate neutrophils in carriers of chronic granulomatous disease. J Infect 6: 96, 1983
12. Page AR, Good RA: Studies on cyclic neutropenia: a clinical and experimental investigation. Am. J. Dis. Child 94: 623 1957
13. Schofield KP, Stone PCW, Stuart J: Quantitative cytochemistry of blood neutrophils in acute myeloid leukaemia. Br. J. Haematol. 54: 261, 1983
14. Scully C, Wilkinson PC: Inflammatory polymorphonuclear neutrophil leukocytes; orientation, chemotactic, locomotor and phagocytic capabilities of neutrophils from the human gingival crevice. J Clin Lab Immunol 17: 69, 1985
15. Skapski H, Lehner T: A crevicular washing method for investigating immune components of crevicular fluid in man. J Periodont Res 11: 19, 1976
16. Van Dyke TE, Levine MJ, Genco RJ: Neutrophil function and oral disease. J Oral Pathol 14: 95, 1985