FLOW CYTOMETRIC APPROACH TO HUMAN POLYMORPHONUCLEAR LEUKOCYTE ACTIVATION INDUCED BY GINGIVAL Crevicular Fluid in Periodontal Disease

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Abstract—In gingival pockets of patients with periodontal disease, polymorphonuclear leukocytes (PMN) are in contact with a peculiar exudate, the gingival crevicular fluid (GCF). Because of the pivotal role played by PMN in periodontal disease, we evaluated the ability of GCF in modulating normal human PMN. GCF was obtained from two gingival sites with severe periodontitis (SP) and two gingival sites with only mild periodontitis (MP) in 12 patients. Purified PMN were exposed to GCF from SP and MP sites and, as a control, to sterile culture medium. GCF activity was evaluated by monitoring the modulation of membrane molecules relevant to cell function. Compared to control medium, GCF from SP and MP sites was able to induce an activation status in PMN evidenced by an increased CD11b (62 ± 9% and 28 ± 7%, respectively) and f-Met-Leu-Phe (56 ± 5% and 31 ± 7%, respectively) receptor expression, with a concomitant reduction of CD62L expression (56 ± 8% and 23 ± 7%, respectively). Thus, reflecting the clinical status, GCF from SP sites was significantly more efficient in affecting PMN than GCF from MP sites. Cell size modifications, evaluated as an additional indicator of PMN activation, were consistent with membrane molecule modulation. The difference in PMN-activating capacity between SP and MP was abrogated by the successful completion of an appropriate periodontal therapy that dramatically improved clinical status. This is the first direct demonstration that GCF from periodontitis has the capacity to activate normal resting PMN and that this capacity reflects the magnitude of the inflammatory process that takes place in the gingiva.
INTRODUCTION

The importance of correct polymorphonuclear leukocyte (PMN) functioning in diverse clinical situations is well established. These cells represent the first line of defense of the host against a variety of pathogens, but can also become, under certain circumstances, key participants in aggravating tissue injury (1, 2).

Periodontal disease is a progressively destructive inflammatory process affecting the supporting tissue of the teeth (3). Extensive data have been produced that demonstrate that PMN are among the main cell types involved in the periodontal disease (3–5). Large numbers of PMN can be found in the gingival pockets of periodontitis patients where they are thought to represent the primary response to plaque bacteria, and evidence has been provided that PMN collected from gingival pockets in periodontitis have the phenotypical and functional features of activated cells (6, 7). A common feature of periodontal disease is the gingival crevicular fluid (GCF), an exudate found in gingival pockets of periodontitis patients that contains a variety of factors derived from both plaque bacteria and the host's inflammatory reaction and that can therefore be regarded as representative of the gingival microenvironment (8–10).

In a previous paper (8) evidence was provided for a modulating activity on chemotaxis of normal resting PMN exerted by GCF. Here we investigated on the possibility that GCF could modulate the functional status of normal resting PMN. Numerous assays for investigating PMN activity have been in use for a number of years. Traditionally, these tests are tedious and time-consuming manual methods. More recently, great interest has arisen about the possibility of testing PMN using flow cytometric techniques: flow cytometry has the unique capability of making reproducible measurements on individual cells in heterogeneous populations at a very fast rate and requires a comparatively smaller amount of cells to be performed (11, 12). Based on this background information, we designed an experimental approach to monitor the capacity of GCF collected from gingival pockets with diverse degrees of involvement in modulating normal resting PMN activity, using flow cytometry and fluorescent probes.

MATERIALS AND METHODS

Patients. Twelve patients (seven males and five females, age range 25–61 yrs) with periodontitis and no sign of peripheral PMN defects were selected. All patients were free of therapy for at least six months. For each patient, GCF was collected from four gingival sites: two with major clinical signs of periodontitis (attachment loss, probing depth >5 mm, and bleeding on probing), hereafter referred to as severe periodontitis (SP) sites, and two with a minor clinical involvement (no attachment loss, no bleeding on probing, and probing depth <3 mm), hereafter referred to as mild periodontitis (MP) sites (9). GCF was collected again from the same sites six to eight weeks