The First Flow Cytometric Determination of Salivary Cell Differential Count

Anđelko Vidović[1], Danica Vidović Juras[2], Ana Cekić-Arambašin[2], Josip Lukač[3], Ante Sabioncello[1], Dragan Dekaris[1]

[1]Institute of Immunology, Rockefellerova 10, HR-Zagreb, Croatia; [2]Department of Oral Medicine, School of Dental Medicine, Gunduliceva 5, HR-Zagreb, Croatia; [3]Department of Oncology and Nuclear medicine, University Hospital Sestre Milosrdnice, HR-Zagreb, Croatia

Introduction

Human saliva is a complex exocrine secretion which contains proteins, hormones, antibodies, ions, bacteria and cells derived from salivary glands, crevicular fluid, tonsils, the oral mucosa, bronchopulmonar and oropharyngeal secretions. Saliva contains important antimicrobial agents and patients with xerostomia are prone to local infections. It has an important role in oral immune system which is a part of an extensive and specialized mucosa-associated lymphoid tissue. Antimicrobial properties of saliva were primarily investigated through its soluble components and little is known about its cellular components. Flow cytometry allows a fast and accurate characterization of cells in human body fluids, so we wanted to know whether cellular composition of saliva can be determined by flow cytometry with special interest to cells involved in local inflammation.

Objectives

- to develop a method to quantify leukocytes and its subsets in paraffin stimulated whole human saliva (SWS) by flow cytometry
- to determine reliability of the method and set reference values by examining healthy individuals
- to set further goals in investigation of the subject

Subjects and methods

The study was performed on samples provided from 8 healthy volunteers (4 males, 4 females, mean age 26.1, SD 1.1). Paraffin SWS samples were taken every morning, three days in a row and analyzed immediately. Peripheral blood samples were taken only on the first day. To lower the amount of debris, cellular aggregates and epithelial cells, samples were filtrated through 47 µm filter pores. Leukocytes were counted in filtrates by conventional microscopy chamber counting. Only two subsequent washing /centrifugation steps were performed, prior to and after the staining. Cells were stained with FITC anti-CD45, PE anti-CD14, PerCP anti-CD20 and APC anti CD3 monoclonal antibodies. Whole blood samples were assessed using lyse/wash standard procedure and stained accordingly for comparison with saliva. Because of low expected frequencies of lymphocytes, flow cytometric acquisition and analysis was performed following rare events detection principles. Based on stains used and FITC threshold gating, we could discriminate major leukocyte populations (granulocytes, monocytes /macrophages and lymphocytes) (Figure 1), as well as T($CD3^+$) and B ($CD20^+$) cells (Figure 2) and measure their percentages. As the presented technique was never performed on saliva, reliability of the method was assessed by calculating between subject (CV_{BS}) and day to day within subject coefficient of variation (CV_{WS}) (Table). Measurement error was assessed by calculating intra-class coefficient of correlation (ICC) for every measured parameter (Table). Saliva values were also compared to peripheral blood values (Figure 3).

Figure 1

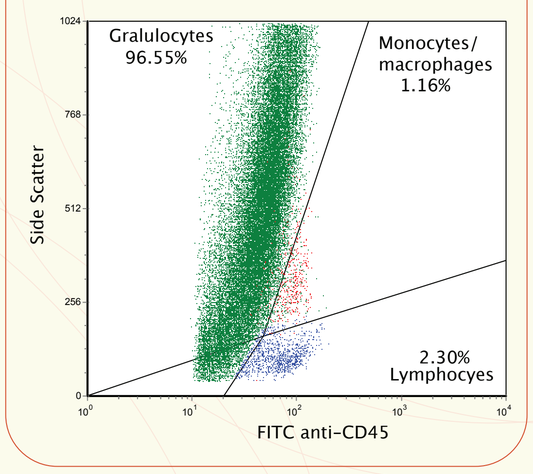
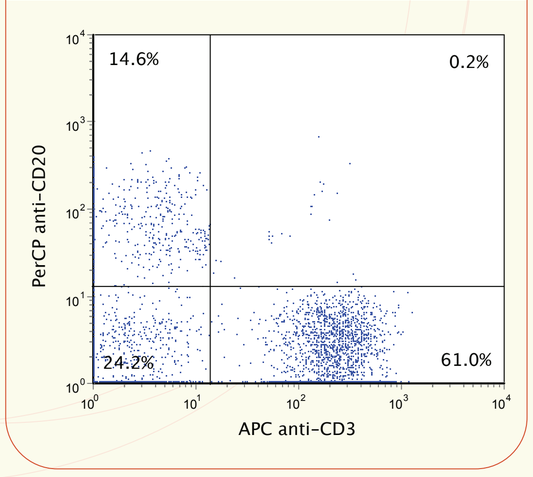


Figure 2

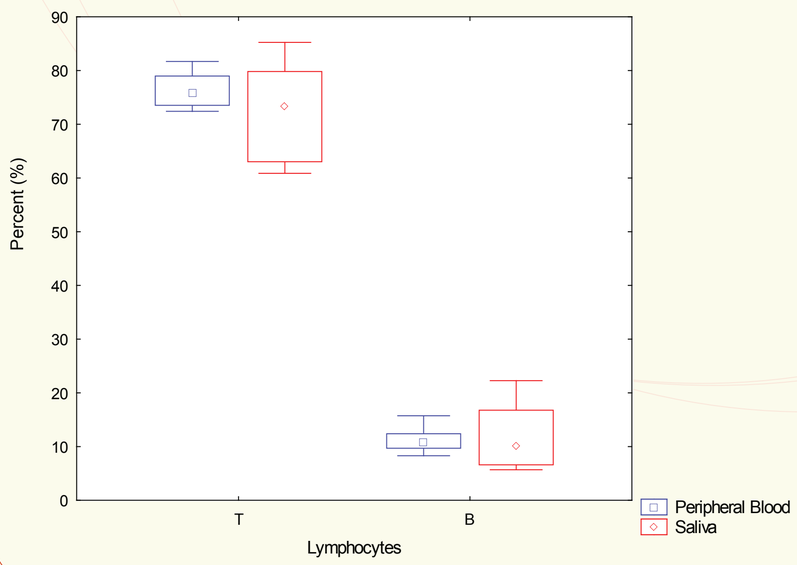


Results

	Peripheral blood			1st measurement			2nd measurement			3rd measurement			ICC[3]	CV_{WS}(%)[4]	CV_{BS}(%)[5]
	Mean	SD[1]	95% CI[2]	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95%CI			
Leukocyte count (x10^6)	-	-	-	1.69	0.43	1.33-2.05	1.69	0.90	0.93-2.44	1.57	0.49	1.16-1.98	0.35	42	39
Granulocytes (%)	56.61*	11.16	47.28-65.94	97.31	1.98	95.65-98.97	97.54	2.34	95.58-99.50	97.51	2.09	95.76-99.26	0.81	1	2
Monocytes (%)	5.06*	3.05	2.50-7.61	1.59	0.93	0.81-2.37	1.43	1.11	0.50-2.36	1.31	0.69	0.73-1.89	0.43	48	64
Lymphocytes (%)	34.28*	8.95	26.80-41.75	1.10	1.22	0.08-2.12	1.03	1.33	0.00-2.14	1.17	1.47	0.00-2.40	0.92	34	122
T cells (%)	76.34	3.46	73.45-79.24	72.32	9.23	64.61-80.03	62.22	6.78	56.55-67.88	59.87	17.77	45.01-74.73	0.02	20	19
B cells (%)	11.20	2.37	9.22-13.18	11.87	6.33	6.58-17.16	10.62	3.30	7.86-13.38	12.82	4.09	9.41-16.24	0.27	27	47

* significant difference between peripheral blood and saliva values ($p<0.001$); [1] standard deviation; [2] 95% confidence interval
[3] intra-class coefficient of correlation; [4] within subject coefficient of variation; [5] between subject coefficient of variation

Figure 3



Conclusions

Flow cytometry can be used to reliably quantify leukocytes and its subsets in paraffin SWS even in healthy subjects.

Granulocytes are predominant leukocyte population in paraffin SWS (92-99%) followed by low frequencies of lymphocytes (0.2-3.4%) and monocytes / macrophages (0.1-4.4%). Percentages of T and B cells are not significantly different from those of peripheral blood.

Because of low frequency of lymphocytes, careful characterization of its subsets using rare event analysis principles is required. Multiparameter flow cytometry techniques are needed for further subtypisation of lymphocytes.

The identification and complete characterization of salivary leukocytes could be used to better understand the pathogenesis of many oral diseases. It could be also useful in diagnosis of oral and systemic diseases.