Evaluation of Apoptosis in Nasal and Buccal Cells of Septic Patients

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Abstract. Background: Inhibition of lung cell apoptosis in the bronchoalveolar lavage (BAL) of septic patients may have a prognostic value for the severity of sepsis. The present study evaluated apoptosis in the nasal and buccal mucosa of septic patients as an alternative and less invasive approach for studying the cells involved in bronchial inflammation. Patients and Methods: A prospective study was designed. Nasal and buccal mucosa brushings were obtained from 20 consecutive septic patients who were admitted to two intensive care units. Twenty-four patients scheduled to undergo surgery for colorectal cancer or laparascopic cholecystectomy were the control group. Apoptosis was evaluated using a TUNEL assay, while BCL-2 and BAX expression were evaluated by immunohistochemistry. Results: Significantly reduced apoptosis in the nasal mucosa of septic patients compared to the control group (p=0.043) was detected only by the TUNEL assay. Conclusion: Reduced apoptosis was found during sepsis in the nasal mucosa in accordance with the reduced apoptosis in the lungs of septic patients. In contrast to septic lungs the underlying mechanism leading to apoptosis in the nasal mucosa was unrelated to the expression of two apoptosis-related genes BCL-2 and BAX.

In the septic lung, death of several cell populations including neutrophils and alveolar macrophages has been observed (1-3). Apoptosis represents an intentional pathway for the removal of these cells, contributing to resolution of the acute pulmonary inflammation (4). In addition, remodeling of certain organ cells (e.g. in the small intestine) has been shown to occur, through apoptosis, in animals during systemic inflammatory response syndrome (1).

In order to examine the effect of apoptosis in lung cell populations, we previously obtained and studied bronchoalveolar lavage (BAL) from critically ill septic patients (5). Spontaneous apoptosis in BAL cells in general, and of alveolar macrophages in particular, was found to be significantly reduced in septic patients compared with non-septic controls. Furthermore, an inverse correlation was found between the percentage of apoptotic alveolar macrophages and the severity of sepsis, suggesting that this approach might have a prognostic value (5). However, there are some disadvantages in obtaining bronchoalveolar lavage repeatedly. It is extremely hard to perform this approach on a sequential or routine basis, since it requires a difficult and expertise-requiring method, which also constitutes an additional disturbance for the critically septic patient.

Apoptosis of nasal and buccal mucosa has not been studied thoroughly in sepsis. It has previously suggested that nasal and buccal mucosa may be representative of the alveolar status in patients with asthma and other chronic obstructive pulmonary disease (6, 7). Eosinophilia in the nasal mucosa correlates well with eosinophilia in the airways of asthmatic patients without symptoms of rhinitis (7). In addition, inflammation present in the nasal mucosa of asymptomatic asthmatics exhibits cellular characteristics also seen in endobronchial biopsies, such as significantly raised numbers of T lymphocytes, CD45RO+ lymphocytes, RFD1+ macrophage-like cells and RFD7+ macrophages. These observations raise the possibility that mucosal sampling may be an alternative and less invasive approach for studying the cells involved in bronchial inflammation (6).

In light of the above, we studied apoptosis in nasal and buccal mucosa samples of septic patients as an alternative and less invasive approach for studying the cells involved in...
the bronchial inflammation. We evaluated apoptosis in the nasal and buccal mucosa of both patients and controls in order to detect any differences in the apoptotic pattern between septic disease and healthy status.

**Patients and Methods**

**Study group.** Nasal and buccal mucosa brushing was obtained from 20 critically ill septic patients who were admitted consecutively to the intensive care unit and the surgical intensive care and trauma unit of a university hospital. The institutional review board for research approved the study protocol. Informed consent was obtained from each patient or a relative. All patients met the clinical criteria for sepsis (Table I) (8). Excluded from this study were patients who had primary lung pathology, had received immunosuppressive agents, had acquired immunodeficiency syndrome, were transfused with >2 units of blood in the 12 h preceding the study enrolment, were younger than 18 and older than 85 years of age, had a malignancy, or were unlikely to survive >24 h. All patients fulfilled the U.S.-European Consensus guidelines for acute lung injury, but none developed acute respiratory distress syndrome (9, 10). The primary site of infection varied: eight patients (40%) had necrotizing pancreatitis, six had fecal peritonitis (30%), four were trauma patients (20%) and two (10%) had catheter-related sepsis. The mean age of the study group was 66.2±2.75 years with a median of 68.5 years. Eleven patients were men and nine were women (male/female ratio: 1:0.84). The patients’ data are shown in Table II. Criteria for systemic inflammatory response syndrome (SIRS) were included in the study. Before nasal and buccal brushing, a thorough investigation confirmed that the patient did not meet any of the exclusion criteria. Clinical and laboratory data as well as Acute Physiology and Chronic Health Evaluation (APACHE) II and Multiple Organ Dysfunction Scores were recorded (11, 12). The obtained PaO₂/FiO₂ and APACHE II values, as well as the pathogenic organisms found are shown in Table II.

**Control group.** Nasal and buccal mucosa brushing was obtained with consent from 24 volunteers with no concomitant lung disease. This group consisted of two subgroups of comparable size, age and sex. The first subgroup (A) contained 11 patients with colorectal cancer and the second subgroup (B) consisted of 13 patients who had scheduled to undergo laparoscopic cholecystectomy. All samples were obtained before their operation. None of the criteria of systemic inflammatory response syndrome (Table I) were met by these patients. None of them had postoperative complications. They were hospitalized for an average of 4.6 days (subgroup A for 6.6 days and subgroup B for 3.0 days). The mean age was 57.2±4.5 years for subgroup A (median 58.0 years), and 58.5±4.2 years for subgroup B (median 64.0 years). In the first subgroup, 5 patients were men and 6 were women, while in subgroup B 6 patients were men and 7 were women (total male/female ratio, including the two subgroups 1:1.2).

Terminal transfer-mediated deoxyuridine 5-triphosphate nick end labeling (TUNEL assay) and immunohistochemical detection of expression of the apoptosis-related genes BCL-2 and BAX. The
TUNEL assay and the BCL-2 detection procedures were performed as described elsewhere (5). The immunohistochemical detection of the BAX gene product was performed with the streptavidin-biotin-peroxidase method using a specific monoclonal antibody (Cadenza Tags Kit, Shandon, Pittsburg PA, USA). Staining for BCL-2 and BAX was classified visually. No staining present in any of the cells was considered negative; any staining seen was considered positive and classified into a grading system consisting of weak staining in <20% of the cells (+), moderate staining in 20% to 50% (+ +) and intense staining in >50% of the cells (+ + +). All slides were evaluated independently by two pathologists.

Statistical analysis. The Fisher’s exact test was used for comparisons between categorical variables. All statistical differences were two-sided, with the significance level set at \( p < 0.05 \).

Results

Subgroup A controls (patients with colorectal cancer) and subgroup B controls (patients scheduled for laparoscopic cholecystectomy) did not have statistically significant differences in TUNEL, BAX and BCL-2 (data not shown). Therefore, the two subgroups were considered homogenous and were combined to form the control group of our study used for comparisons with the septic cohort (Table III).

The TUNEL assay on nasal mucosa samples indicated apoptosis in 4 out of 20 septic patients, and 11 out of 24 controls, a statistically significant difference \( (p=0.043) \). This significant reduction in apoptosis was not seen with either the BCL-2 or the BAX expression study of nasal mucosa (Table III). Finally, apoptosis was not reduced in buccal mucosa of septic patients compared to controls (Table III).

Discussion

Apoptosis has been shown to be an important mechanism of cell death in animal models of sepsis and endotoxemia (13, 14). In human sepsis, apoptosis is the key regulator of the balance between pro- and anti-inflammatory processes (15, 16). Different cell populations, such as leukocytes, vascular endothelial cells, parenchymatous or mesenchymatous cells may die by apoptosis, contributing to multiple organ dysfunctions (13-16). It is not clear whether apoptosis is beneficial or detrimental to the host. Initially, its role is beneficial, because during sepsis it results in the removal of toxic products that may severely injure several organs. However, there is a critical limit beyond which prolonged apoptosis may lead to tissue destruction, organ failure and death (5).

It has been suggested that the nasal mucosa may possibly represent the alveolar status in non-septic patients (6, 7, 17), and it is easily accessible for sequential sampling without causing a major disturbance to patients. In this study, we evaluated the effect of sepsis on the apoptosis of the upper respiratory tract (nasal mucosa). A statistically significant decrease of apoptosis in the nasal mucosa of septic patients was detected with the TUNEL assay in comparison to non-septic controls. Still, the apoptotic signal and the corresponding mechanism seem to be different than those we previously observed in BAL fluid (5). The BCL-2 and BAX protein expression was not significantly different in the nasal mucosa between septic patients and controls in contrast to the findings from BAL, where there is evidence suggesting that BCL-2 may be a regulator in the late stage of apoptosis in lung cells (5). This is an important difference in the apoptotic profile of the upper and lower respiratory tract in sepsis. These observations indicate that there is indeed inhibition of apoptosis (as already seen in alveolar macrophages), but in nasal mucosa the BCL-2/BAX system does not play a major role in regulating apoptosis.

In conclusion, reduced apoptosis was found during sepsis in the nasal mucosa in accordance to the reduced apoptosis in the lung of septic patients. Nevertheless, the underlying mechanism leading to apoptosis in the nasal mucosa was unrelated to the expression of the two apoptosis-related genes BCL-2 and BAX, in contrast to septic lungs. This different underlying mechanism of apoptosis needs further

<table>
<thead>
<tr>
<th>Method</th>
<th>Study group</th>
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<th>Significance</th>
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<tbody>
<tr>
<td>a) TUNEL assay</td>
<td>Nasal mucosa</td>
<td>(+) 4, (-) 16</td>
<td>0.043</td>
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<tr>
<td></td>
<td>Buccal mucosa</td>
<td>(+) 7, (-) 13</td>
<td>N.S.</td>
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<tr>
<td>b) BCL-2 expression</td>
<td>Nasal mucosa</td>
<td>(+) 10, (-) 10</td>
<td>N.S.</td>
</tr>
<tr>
<td>c) BAX expression</td>
<td>Nasal mucosa</td>
<td>(+) 3, (-) 17</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>Buccal mucosa</td>
<td>(+) 5, (-) 15</td>
<td>N.S.</td>
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+, positive; –, negative; N.S., not significant.
investigation to be determined. Consequently, nasal mucosa brushing may not provide an alternative way of determining and monitoring the apoptotic state of the lung. Its clinical impact and correlation with the outcome is not established according to our results and additional studies are required to evaluate such a relationship.

References


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