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Cell populations and adhesion molecules expression in conjunctiva before and after bone marrow transplantation

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Abstract

We were interested to analyse the composition of the cellular infiltrate and adhesion molecules expression in the conjunctiva before and at least one hundred days after autologous and allogenic bone marrow transplantation (BMT) and its relation with the presence of dry eye. We used immunohistochemistry on cryopreserved human conjunctiva with monoclonal antibodies to T-lymphocytes (CD3, CD4 and CD8), B-lymphocytes (CD19), macrophages (CD14), natural killer cells (NK, CD57), intercellular adhesion molecule 1 (ICAM-1), E-selectin, vascular cell adhesion molecule-1 (VCAM-1), lymphocyte function associated antigen-1 (LFA-1), very late antigen-4 (VLA-4), interleukin 2 receptor (IL2r, CD25) and HLA-DR. Our autologous recipients had no graft-versus-host disease (GVHD) but allogenic patients had chronic GVHD. After autologous BMT the conjunctiva had significantly more: (1) T lymphocytes (CD3+, CD4+, CD8+) in the epithelium; (2) CD4+ and CD14+ cells in the stroma; and (3) VLA-4 expression in the stroma than before BMT. After allogenic BMT, the conjunctiva exhibited a significant increase of: (1) CD3+ and CD14+ cells in the epithelium; (2) T lymphocytes (CD3+, CD4+, CD8+) and CD14+ cells in the stroma; and (3) VLA-4 and LFA-1 expression in the stroma than before BMT. After the engraftment, the comparison between autologous and allogenic recipients revealed that: (1) there were no significant differences in adhesion molecule expression; (2) the epithelium of autologous recipients had significantly more CD3+ cells; and (3) the stroma of allogenic patients had significantly more CD3+ and CD8+ cells. Among allogenic recipients, CD14+ cells were significantly increased both in the epithelium and in the stroma of patients with signs or symptoms of dry eye in comparison with patients without ocular involvement. Additionally, those having keratoconjunctivitis sicca (KCS) had CD4/CD8 ratios significantly higher than those without KCS.

In conclusion, in the conjunctiva after autologous BMT a subclinical cell mediated immune reaction seems to take place. The conjunctivitis of chronic GVHD is complex, with T cells and macrophages dramatically contributing to the process.

Keywords: bone marrow transplantation; graft-versus-host disease; dry eye; keratoconjunctivitis sicca; conjunctiva; lymphocytes; adhesion molecules

1. Introduction

Stem cell transplantation (STC) is used to treat a variety of malignant and non-malignant diseases (Bortin and Rim, 1989; Thomas, 1989). Donor T lymphocytes are generally agreed to be the primary aggressors in establishing graft-versus-host disease (GVHD) (Van Els et al., 1990a,b,c). The afferent stage of GVHD involves recognition of minor and major histoincompatibilities by donor T cells (Martin, 1991). Then during the efferent stage, donor cytotoxic lymphocytes attack host cells. However, the combination of factors that renders cells targets of GVHD has not yet been clarified (Sale et al., 1998). On the other hand, cellular infiltrate composition and adhesion molecules expression in organs affected by GVHD varies depending

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on the target organ analysed (Dilly and Sloan, 1985, 1987; Elliot et al., 1988; Leskinen et al., 1992; Forbes et al., 1996).

GVHD can be divided into two entities: acute and chronic GVHD. Although improvements have been made in the prevention of acute GVHD, these advances have not resulted in a concomitant decrease of chronic GVHD (Vogelsang, 2001). In fact, chronic GVHD remains the most common late complication of allogenic stem cell transplantation (Vogelsan, 1992, 2001). Chronic GVHD is a major complication of STC (Bortin and Rim, 1989; Wingard et al., 1989; Tanaka et al., 2000) that generally occurs after the first 100 days following transplantation, affecting approximately 20–50% of patients who survive beyond this time (Jack et al., 1983). Chronic GVHD is a heterogeneous disease that involves a much wider range of organs than acute GVHD, among which is the eye. It has a variable clinical picture (Wick et al., 1985) closely mimicking spontaneously occurring connective tissue disease, notably scleroderma and Sjögren syndrome (Gratwhol et al., 1977; Lindahl et al., 1988).

Ocular complications of GVHD occurs in about 60–80% of the patients. The most frequent ocular manifestation is keratoconjuntivitis sicca (KCS) (Franklin et al., 1983; Hirst et al., 1983). The incidence of dry eye in chronic GVHD range from 19–84% (Hirts et al., 1983; Calissendorf et al., 1989; Livesey et al., 1989; Arocker-Mettinger et al., 1991; West et al., 1991; Tichelli et al., 1996; Ogawa et al., 1999).

Dry eye is included among the features for extensive chronic GVHD classification (Sullivan et al., 1999); however, physiopathology of dry eye in GVHD have been an object of controversy. Some authors have attributed it to therapeutic regimens related to BMT and/or GVHD prophylaxis (Franklin et al., 1983; Calissendorf et al., 1989; Livesey et al., 1989). In contrast, some others include the conjunctiva as an immunological target of GVHD (Sale et al., 1981) and consider dry eye in GVHD not related to therapeutic regimens (Abesada-Terk et al., 1990; Bray et al., 1991; Menucci et al., 1997).

Interest in dry eye in chronic GVHD has been mainly focused on clinical manifestations (Gratwhol et al., 1977; Franklin et al., 1983; Jabs et al., 1989; Livesey et al., 1989; Arocker-Mettinger et al., 1991; Bray et al., 1991; West et al., 1991; Tichelli et al., 1996; Lawley et al., 1997; Menucci et al., 1997; Ogawa et al., 1999;) and on the lacrimal gland (Sale et al., 1981; Jack et al., 1983; Ogawa et al., 2001). Only two reports examined the T-lymphocyte subsets of the conjunctiva during chronic GVHD. They included one and six patients, respectively (Bhan et al., 1982; Sainz de la Maza et al., 1996). However, analysing what differentiate conjunctiva from patients with and without KCS in chronic GVHD has not been attempted to date.

The aforementioned reasons led us to undertake the present study.

2. Material and methods

2.1. Patients

Patients who underwent BMT between September 1994 and December 1995 plus four additional patients engrafted before this period were selected for the study. The conditioning regimen varied according to the type of hematological disease and included different combinations of Busulfan, Cyclophosphamide, Melfalan and Methylprednisolone. Patients with clinical criteria and skin biopsies diagnostic of GVHD (Thomas et al., 1975a,b) were referred from the Department of BMT at the Pro-Sangue Hemocentro Foundation of Sao Paulo to the Department of Ophthalmology, Paulista School of Medicine, Federal University of Sao Paulo, for ocular examination. The criteria for patient eligibility were: age more than 10 years, platelet count more than $20 \cdot 000$ cu.m.m^{-}, no previous ocular pathologies, no acute conjunctival or corneal disease at clinical examination and written informed consent to participate in this study. Tissues were studied in accordance with the Helsinki Declaration. All donors had given permission to use their tissues for research.

2.2. Ocular examination and biopsy procedure

Ocular examination, clinical diagnostics test for dry eye, and conjunctival biopsies were performed in the pre-transplant and post-transplant period. Pre-transplant examination and biopsies were done from -30 to -10 days before bone marrow transplantation (BMT) to avoid the effect of drugs used as a conditioning regimen. The post-transplant procedures were done after a minimum of 100 days after BMT. In every visit, patients underwent a complete ocular examination. The ocular surface was evaluated using the Rose Bengal test, the Schirmer test with topical anesthesia and the Tear Breakup Time (BUT). Test were performed and interpreted as proposed in the literature (Jones, 1966; Norm, 1969; Van Bijsterveld and Hollan, 1969). The presence of KCS was considered a criterion of ocular involvement by GVHD. The diagnosis of KCS was based on the presence of Rose Bengal staining greater than 3 with concomitant Schirmer test results less than or equal to 5 mm in 5 min with anesthesia. Signs and symptoms considered to be related to dry eye but not diagnostic of KCS were burning, foreign body sensation, itching, ocular pain, mucus discharge, tearing, corneal filaments and conjunctival hyperemia.

Ocular biopsies were performed under identical conditions in all cases. For those patients having a biopsy before and after BMT, tissues were taken from the left eye before BMT and from the right eye after BMT. Specimens sized 4×4 mm were harvested under topical anesthesia and subconjunctival injection of 2% lidocaine with epinephrin from the superior bulbar conjunctiva adjacent to the limbus was done.