GESINAS - ImmunoTools Award 2015

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Influence of Immunological Biomarker Profile in the Therapeutical Success of Autologous Melanocyte and Keratinocyte Cell Suspension Transplants in patients with Stable Vitiligo

Vitiligo is an autoimmune disease characterized by the appearance of pigmented lesions in the epidermis as a result of progressive loss of functional melanocytes in the affected regions. It affects about 0.5 to 2.0% subjects around the world, leading to their decreased quality of life by stigmatization, especially in mulatto and black population[1,2]. The appearance of the lesions is due to a combination of environmental and genetic conditions involving biochemical and immunological factors, which result in oxidative stress and subsequent activation of melanocytes destruction by immunological cells[3,4,5].

The most accepted hypothesis for the development of the disease is that melanin production can trigger an exacerbated cellular stress, which is further aggravated by environmental conditions. This stress is presented as an excessive accumulation of intracellular reactive oxygen species, which acts as cell damage signal, attracts dendritic cells and primes $CD8^+$ T cells, starting the autoimmune process[6].

Furthermore, it has been shown that the inflammatory profile found in the injured tissue is characterized by increased infiltration of macrophages and TNF-α secretion and IFN-γ by $CD4^+$ T cells. In parallel, IL-6 and IL-8 are secreted by melanocytes[6,7]. Phenotypic changes in perilesional melanocytes such as increased HLA-DR and ICAM-1 expressions have also been described[8].

Considering the important differences between damaged and healthy skin areas, especially in melanocytes, it was developed a surgery treatment employing autologous melanocytes and keratinocytes cell suspensions. In this treatment, cells in the pigmented region of the patient's skin are transplanted to the affected areas to induce their repigmentation. This procedure has been performed routinely at Clari Dermatology and Allergy Clinic by the dermatologist Dr. Daniel Gontijo Ramos in partnership with the immunologist Dr. Mariana Gontijo Ramos, and the physiotherapist Dr. Camila Gontijo Ramos.

In the last two years more than 40 patients underwent the transplantation by one of us (DGC), proving its safety and efficiency[9]. Although promising, this technique still

presents very variable results may be owing to the immune status of patients before and after transplantation as well as to the content of the explant. Therefore, the goal of this study is to evaluate the influence of immunological biomarker profile in the therapeutic success of autologous melanocytes and keratinocytes in patients with stable vitiligo.

For this purpose, 5 (five) mL of peripheral blood will be collected from 40 patients with stable vitiligo as follows: immediately prior to transplantation (D0), seven days (D7) and ninety days (D90) after transplant to assess the cellularity profile in peripheral blood through immunophenotyping of circulating leukocytes (CD3, CD4, CD8, CD14, CD16, CD19, CD25, CD69, HLA-DR, CD62L, CD54, CD18, CCR2, CCR4, CXCR3, CXCR4 e CCR5), evaluation of serum cytokines and chemokines, and other soluble factors (IL-1-β, IL- 2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, TNF-α, IFN-γ, MIF, CCL2, CCL7, CXCL-9, IL-8, MCP-1, MIG, IP-10, MCP-3, sICAM-1). In parallel, we will evaluate the humoral immune response (anti-melanocyte antibodies). Furthermore, about 20% of the explant cell suspensions will be collected to evaluate cell viability, melanocytes/keratinocytes ratio, and their immunophenotypic profile (Tyrosinase-TRYP, Citokeratin-K14, ICAM-1 e E-cadherin). It will be also collect a skin fragment of the perilesional skin area obtained by a 3 mm punch to characterize the inflammatory infiltrate in this skin region. At the end of the project, we hope to find appropriate biomarkers that contribute to establish success criteria/treatment failure in vitiligo patients, which may will improvement the proposed transplantation.

The products supplied by ImmunoTools will be important for the selection and quantification of cell populations involved in the disease, immunophenotyping, and quantification of serum cytokines and chemokines.

Charity Activities for GESINAS-part of the award:
In this work, it will be included annually at least 20 patients from the Charity Hospital of Belo Horizonte, who will receive no cost treatments by autologous melanocyte and keratinocyte transplantation. This procedure will be coordinated by the dermatologist members of our research group, Dr. Jackson Machado Pinto, Dr. Maria Silvia Laborne Alves, and Dr. Daniel Ramos Gontijo.

It is important to mention that our team acts on charity projects at the hospital aiming to health promotion and prevention of the Brazilian population. We coordinate the Annual Campaign of the National Day Prevention of Skin Cancer, in which medical volunteers dermatologists proceeding a unpaid clinical evaluation of people with suspected skin lesions.

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