

A Review of IMMUNEPOTENT CRP, A Modifier of Biological Response: Efficacy and Current Practice

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ABSTRACT

IMMUNEPOTENT CRP is a biological response modifier with intrinsic antioxidant, anti-inflammatory, and antitumor properties. In the following review, we have summarized almost 20 years of research with this bio-compound regarding its obtention method, action mechanism, molecular target and in vitro, preclinical and clinical research.

Keywords: Immunepotent CRP; Dialyzable Bovine Spleen Extract; Antioxidant; Anti-Inflammatory; Antitumoral; Damage-Associated Molecular Patterns

Introduction

The immune system is determinant for survival, and a delicate balance is necessary to keep control of its response, and to avoid over-response and non-response toward self and non-self-antigens. The immune system is an integrative network formed by different types of cells and soluble mediators only produced in doses needed by each body for an optimal function [1]. During pathological states, the organism occasionally needs treatment to restore homeostasis. The biological response modifiers refer to substances that interact with the host immune system and modify its response [2]. Biological response modifiers are used for the treatment of diseases or to reduce side effects caused by aggressive treatments, such as treatments against cancer or autoimmune diseases [2]. These modifiers are classified based on their activities as vaccines and antibodies that provide antigen-specific activity and may have a target direct effect or compounds that stimulate the immune system without antigenic specificity [3]. The focus of this review is on the biological response modifier IMMUNEPOTENT CRP, a dialyzable bovine spleen extract with the activity of transfer factor, mainly used as a cancer therapy adjuvant that also reduces chemotherapy

side effects and has intrinsic antioxidant and antitumor properties. Our research group has worked with IMMUNEPOTENT CRP at *in vitro*, preclinical and clinical levels for almost 20 years.

IMMUNEPOTENT CRP Obtention

The bovine dialyzable leukocyte extract is obtained from the spleen, that can contain specific transfer factor activity or not. The extract consists of a mixture of active small (12kDa or less) immunomodulating agents. Transfer factor is a substance capable of transferring antigen-specific information from an immunized donor to a naïve recipient [4]. Therefore, this property depends on whether the bovine was previously immunized against a determined antigen or microorganism. The bovine spleen (with an approximate weight of 900g) was chosen for the extract because of a great amount of immune system cells that contains and the amount of raw material available [5]. This product is manufactured and distributed in Mexico by the enterprise LONGEVEDEN S.A de C.V. under the trademark IMMUNEPOTENT CRP. Briefly, the spleen is homogenized and later dialyzed against bi-distilled water for 72h. The dialysis is clarified using a filter with a 0.2 μ m pore size,

pasteurized at 65°C for 3h, and lyophilized or not depending on the commercialization requirement.

Biological Properties

IMMUNEPOTENT CRP contains many active substances with functional activities; this diversity of function allows many applications of our product in the human health field. To date, our main focus is the antioxidant, anti-inflammatory and anti-cancer properties of our bio-compound, which have been studied *in vitro* or *in vivo*, as described below. The reactive oxygen species or free radicals (ROS) are naturally produced by oxidation reactions in the organism. In response, the body counts with an efficient system of antioxidant control, but when the balance between oxidants and antioxidants fail, excessive amounts of ROS are released without control and can result in diseases such as cancer, and chronic inflammation, diabetes, arthritis, and premature aging [6]. The use of antioxidants for the treatment and prevention of distinct diseases is a popular trend based on scientific evidence. *In vitro* studies demonstrated that IMMUNEPOTENT CRP is an antioxidant because it induces reduction of MTT by itself; in addition to this, decreases NO and TNF- α levels, increases antioxidant molecules and decreases I κ B phosphorylation and p50 and p65 NF κ B DNA binding in LPS-stimulated human [7] and murine [8] macrophages.

Furthermore, IMMUNEPOTENT CRP regulates the production of IL-6 and IL-10 and the expression of pro-inflammatory cytokines (IL-1 β , IL6, IL-10, TNF- α , IL-12, IFN- γ) at a transcriptional level [7,8]. In a murine endotoxic shock model, IMMUNEPOTENT CRP improved the survival of mice with LPS induced toxic shock, modulating the pro-inflammatory cytokine at transcriptional and translational levels [9]. All these findings correlate with the beneficial results observed with the use of IMMUNEPOTENT CRP in rescuing newborns from septic shock, where the 100% of the newborns treated survived [10]. Therefore, we proceeded with a clinical trial to evaluate the anti-inflammatory potential of IMMUNEPOTENT CRP in patients undergoing third molar extraction surgery. Ibuprofen was used a standard therapy control. Both treatments decreased pro-inflammatory cytokines and swelling [11].

IMMUNEPOTENT CRP also activates NrF2, a transcription factor part of the antioxidant response element pathway that increases the antioxidants glutathione peroxidase, catalase, and superoxide dismutase enzymes and eliminates ROS, in mice undergoing 5-fluorouracil chemotherapy. Myelotoxicity is a dose-limiting effect of many chemotherapeutic regimens; therefore, compounds with antioxidant activity that do not compromise chemotherapy efficacy are desirable. Additionally, IMMUNEPOTENT CRP increased committed cell lineage populations, such as leukocytes (CD45+), granulocytes (CD11b+ Gr-1+), and erythrocytes (CD71, Ter119). The mice also presented normal hematological parameters (WBC and RBC) [12,13]. It is reasonable to speculate that the anti-inflammatory and antioxidant activities of IMMUNEPOTENT CRP

are mediated by its capacity to modulate inflammation and ROS through NF κ B, I κ B and NrF2 pathways.

Besides its chemo-protective activity, IMMUNEPOTENT CRP has cytotoxic effect against several cancer cell lines, including MCF-7, BT-474, MDA-MB-453 (breast cancer), A-427, Calu-1 (lung cancer), U937 (leukemia), and L5178Y (lymphoma) [13], B16F10 (melanoma) [14], K562, MOLT-3 (human leukemia) [15], and HeLa (cervical cancer) [16] *in vitro*; also, toxic doses for cancer cells do not affect human PBMC [13] human monocytes, and murine peritoneal macrophage [15]. Furthermore, IMMUNEPOTENT CRP possesses antitumor activity against murine lymphoma [16] and melanoma [17] in a dose-dependent manner. The search for novel drugs is still a priority goal for cancer therapy due to the inefficiency, high toxicity, adverse effects and development of resistance to chemotherapeutic drugs. Therefore, new drugs destined for cancer treatment should induce fewer side-effects and/or have greater therapeutic benefit [18].

It is worth mentioning that, although administration of this product for humans is by the oral and parenteral route. The maximum subcutaneous or intramuscular administration dose for a mouse is 5 units. Higher doses caused too much discomfort and severe pain. It is reasonable to assume that this is the reason why complete tumor regression was not achieved in the murine models [16,17]. On the contrary, with 50 units administered to dogs by the intravenous route, there are no signs of discomfort or pain [data not published yet]. Also, IMMUNEPOTENT CRP has been administered to human patients with lung and breast cancer undergoing chemotherapy or/and radiation therapy. This combined therapy resulted in improved life quality and immunological parameters, and tumor reduction, in comparison to the group not receiving IMMUNEPOTENT CRP, indicating that its administration as an adjuvant in cancer treatment is beneficial for the patients [18,19]. Additionally, IMMUNEPOTENT CRP is administered as an adjuvant in the treatment of allergies, asthma, herpesvirus I and II, coccidioidomycosis and diabetes with promising results in the current clinical practice (data not shown).

Molecular Targets

IMMUNEPOTENT CRP induces apoptosis in breast cancer cells by suppressing the AP-1 DNA-binding and modulating NFATx, NFATc, NF- κ B, c-Jun and c-Fos at a transcriptional level [20]. In melanoma cells, IMMUNEPOTENT CRP induces apoptotic and antiangiogenic effects modulating the production of vascular endothelial growth factor [VEGF] *in vivo*, preventing metastasis and delaying tumor development, and increasing the survival period of tumor-bearing mice [12]. In leukemia cells, an interesting effect was observed: low doses of IMMUNEPOTENT CRP induce immature leukemic cell differentiation to the monocyte/macrophage lineage with M2 phenotype, or to the megakaryocyte lineage [CD42+]. It also induces cell cycle arrest in the S and G2/M phases and decrease of the nitric oxide levels. Induction of cell differentiation is a

highly desirable effect for leukemia treatment since this approach prevents the leukemic blast crisis with high proliferation rates [14]. The administration DAMPs (Damage-associated molecular patterns) rich cell lysates derived from B16F10 cells treated with IMMUNEPOTENT CRP or the combination with oxaliplatin prevented melanoma growth in mice, on the contrary, oxaliplatin treatment alone did not. Immunogenic cell death induction correlates with tumor prevention and long-term remission [17].

More recently, we evaluated the effect of IMMUNEPOTENT CRP over HeLa cells, observing cell cycle arrest in the G2/M phase, mitochondrial damage, and ROS mediated caspase-independent cell death [15]. This finding is important because, despite the development of preventive vaccines, HPV remains a common cancer cause among women and new therapies are needed [22]. To corroborate that the oral route administration of IMMUNEPOTENT CRP does not decrease its biological properties, our bio-compound was treated with hydrochloric acid to lower the pH (2.0) and exposed to the activity of gastrointestinal enzymes [proteases, nucleases, polysaccharide-degrading enzymes or lipase]. After enzymatic treatment, we modified pH to neutral levels (7.0) and enzymes were inactivated by heat (100°C for 15 minutes). Our results indicated that IMMUNEPOTENT CRP biological properties are stable after exposition to low pH levels and enzymatic cleavage. Furthermore, the treatment with proteinase K increased its cytotoxic activity. Therefore, we evaluated the antitumor activity of IMMUNEPOTENT CRP after treatment with proteinase K, demonstrating an improvement of the antitumor activity in a murine lymphoma model. This finding is an opportunity to optimize the formulation of IMMUNEPOTENT CRP [16].

Conclusion

IMMUNEPOTENT CRP has been used in Mexico as an immunomodulator and a healing agent for neoplastic, inflammatory, and immunity (autoimmunity and immunodeficiencies) disorders. Its therapeutic potential has been demonstrated as an antioxidant and a concomitant/adjuvant therapy to treat human cancer patients. This bio-compound can modulate multiple molecular targets, is free of adverse/toxic effects when administered by the oral or parenteral route to people and dogs. Furthermore, is simple to produce, formulate, and administrate. IMMUNEPOTENT CRP is a promising agent ready to be exploited as a complementary treatment for the preventive and therapeutic management for immune system or antioxidant related diseases.

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