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Dehydroepiandrosterone modulates T-cell response after major abdominal surgery

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ABSTRACT

Background: The immune balance controlled by T-helper (Th)1 and Th2 cells is critical in protecting the host from pathogenic invasion, and its imbalance may increase susceptibility to infection in patients undergoing major surgery. The differentiation of naive T cells to Th1 and Th2 cells is largely driven by cytokines. In addition, steroid hormones have been shown to affect Th1/Th2 balance, particularly in autoimmune diseases. The regulation of Th1/Th2 balance in patients undergoing surgery and its potential clinical relevance remain unclear.

Materials and methods: Blood samples were obtained from patients both before and 2 h after major abdominal surgery. Peripheral blood mononuclear cells were isolated and cultured in wells coated with either anti-CD3 (direct T-cell stimulation) or phytohemagglutinin (PHA) (indirect T-cell stimulation), with or without 10^{-5} M dehydroepiandrosterone (DHEA). The release of interleukin (IL)-2, interferon gamma, and IL-10 was measured by an enzyme-linked immunosorbent assay, and the expression of CD4, CD8, and CD69 was determined by flow cytometry.

Results: DHEA decreased the release of IL-2 and IL-10 in directly (anti-CD3) and indirectly (PHA)-stimulated T cells from postoperative samples, whereas the release of interferon gamma in PHA-stimulated T cells was not affected. The distribution of CD4/CD8 was not significantly different after surgery or DHEA. DHEA was associated with a decrease in the expression of the activation marker CD69 on $CD4^+$ T cells, whereas the activation of $CD8^+$ T cells remained unchanged.

Conclusions: These results demonstrate that DHEA plays a critical role in controlling Th1/Th2 balance in the immediate postoperative period. Attenuation of both the Th1 and Th2 responses has been suggested to have immunoprotective effects. The role of DHEA in the regulation of Th1/Th2 balance in patients undergoing major abdominal surgery may, therefore, also be of significant clinical relevance and warrants further investigation.

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1. Introduction

The protective immunity of the host depends on multiple factors, including the activation and recruitment of polymorphonuclear neutrophils, monocytes, and macrophages; an intact macrophage—T-cell interaction; adequate T-helper (Th)1/Th2 conception of Th-cell activation; and an appropriate cytokine balance [1]. The systemic inflammatory response syndrome after major abdominal surgery is associated with a massive, unbalanced release of cytokines [2–5]. This prolonged activation of the immune response may increase the risk of postoperative infections [6,7]. The normal immune response is largely dependent on the activation of two functionally distinct subsets of mature $CD4^+$ Th cells, which play a central role in cytokine synthesis [8]. Antigen-experienced $CD4^+$ T cells are subdivided into Th1 and Th2 cells based on their production of particular cytokines, with Th1 cells producing interferon gamma (IFN-γ) and interleukin (IL)-2, and Th2 cells producing IL-4 and IL-10. After surgery, the so-called "proinflammatory" Th1-mediated pathway has been shown to be temporarily depressed, whereas the so-called "anti-inflammatory" Th2-mediated pathway remains unaffected or even upregulated. This results in a shift of the Th1/Th2 balance toward Th2 dominance (Th2 shift), which in turn has been associated with an increased susceptibility to postoperative infections [9,10]. Furthermore, T cells can augment immune response locally and systemically [11,12]. An early Th1 response after surgery has been hypothesized to support the inflammatory response by producing IL-2 and IFN-γ, which might reflect the magnitude of the initial inflammation [13].

Dehydroepiandrosterone (DHEA) is a pregnenolone derived from C19 steroid and is synthesized in the zona reticularis of the human adrenal gland by the steroidogenic enzyme CYP17A1 [14]. It is the major circulating steroid hormone in humans [15,16]. A number of studies have now revealed that DHEA is a potential regulator of immune function, eliciting immune stimulatory effects *in vivo*, and often counteracting the immune-suppressive effects of glucocorticoids [17]. Numerous previous studies have investigated the effect of DHEA on cytokine secretion [18,19]. DHEA is reported to be a potent enhancer of IL-2 secretion from Th1 cells. However, in contrast to its promotion of cytokine secretion from Th1 cells, DHEA negatively regulates the production of the Th2 cytokines IL-6 [19,20] and IL-10 [21]. Although a fall in circulating DHEA levels may result in a dysregulated cytokine balance with reduced Th1 cytokine production and increased Th2 cytokine production, DHEA supplementation was shown to reverse these alterations in cytokine production [18–21]. In addition, the administration of DHEA restored depressed cell-mediated immune responses in a mouse model of traumatic hemorrhage [22] and improved survival after the subsequent induction of sepsis [16]. Moreover, DHEA prevented splenocyte dysfunction in mice after thermal injury [23]. Similar to these experimental results, in *in vitro* studies DHEA was shown to normalize the proinflammatory release of cytokines from human peripheral blood mononuclear cells (PBMCs) after major abdominal surgery [24].

Although these studies collectively suggest that DHEA may have immune enhancing effects, its influence on T-cell–mediated immune responses in the context of an unbalanced cytokine release after major abdominal surgery remains unknown. Therefore, the aim of this study was to determine the effect of DHEA on Th1 and Th2 cytokine responses and its influence on T-cell subpopulations in patients undergoing major abdominal surgery.

2. Material and methods

2.1. Patient recruitment

The present study was a prospective, *in vitro* experimental study approved by the Human Subjects Ethical Committee of the Ludwig-Maximilians University, Munich. This study was performed according to the ethical principles of the Helsinki Declaration. Written consent was obtained from all patients preoperatively. The study included a total of 11 patients (seven males and four females) with age ranging from 39–85 y (mean age 62.6 y). All patients were undergoing elective, major abdominal oncologic surgery including colorectal resections (30%), liver resections (50%), and other abdominal procedures such as pancreatic surgery (20%). Exclusion criteria included immunosuppressive therapy, age <18 and >85 y, polytrauma, gravidity, multimorbidity, preexisting chronic alcohol abuse, heart insufficiency, cirrhosis of the liver, portal hypertension, and respiratory insufficiency.

2.2. Blood collection and PBMC isolation

Forty milliliters of peripheral venous blood was obtained in sterile heparinized tubes preoperatively and 2 h postoperatively. Blood samples were centrifuged for 8 min at 520*g* and plasma was collected. The remaining blood cells were diluted (1:2/0.75×) in the Hanks balanced salt solution; PBMC isolation was performed immediately by standard Ficoll density gradient centrifugation at 950*g* (2000 rpm) at room temperature for 20 min. The PBMC layer was removed and washed two times in the Hanks balanced salt solution at 4°C for 15 min at 450*g* (1500 rpm). After this, the cells were resuspended in culture medium (Roswell Park Memorial Institute 1640 + 10% fetal calf serum with 1% gentamicin); cell counts were performed using the Neubauer counting chamber with trypan blue exclusion for testing cell viability [24].

2.3. Cell culture conditions

Before cell culture, wells were coated with the monoclonal antibody anti-CD3 at a concentration of 10 μg/mL for 90 min or phytohemagglutinin (PHA) at a concentration of 1 μg/mL. The anti-CD3 was produced and purified from the OKT3 clone (gift from Dr Natasha Engel, Chiles Research Foundation at the University Hospital Grosshadern, Munich). PBMCs were incubated at a final cell count of 1.0×10^6 cells per well with Roswell Park Memorial Institute 1640 medium and 10% fetal calf serum (at 37°C, 5% CO_2, and 90% humidity) with or