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**These research projects, undertaken in partial fulfillment of the requirements for the M.D. degree at Sackler Faculty of Medicine, Tel Aviv University in 2008–2009, were considered the most outstanding of the graduating class**

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### Microcytic-hypochromic anemia in children: differentiating iron deficiency anemia, $\alpha$ -thalassemia trait and $\beta$ -thalassemia trait with accessible laboratory parameters

N. Shilo-Pugatch<sup>1</sup> and H. Tamar<sup>2</sup>

<sup>1</sup>Department of Internal Medicine A, Rabin Medical Center and <sup>2</sup>Hematology Unit, Schneider Children's Medical Center of Israel, Petah Tikva, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel [noyas3@clalit.org.il]

**Background:** Microcytic-hypochromic anemia is common worldwide in children as well as in adults. The main causes are iron deficiency anemia (IDA),  $\alpha$ -thalassemia ( $\alpha$ TT) trait (carrier), and  $\beta$ -thalassemia ( $\beta$ TT) trait. Although the clinical and laboratory characteristics are similar for all three, their differentiation is important because each requires a specific treatment and has different sequelae if left untreated. Iron deficiency, even without anemia, results in neurologic developmental damage; thalassemia trait places affected individuals at risk of having a child homozygous for a debilitating and life-threatening disease. Most of the research so far on microcytic hypochromic anemia has concentrated on differentiating IDA from  $\alpha$ TT in the adult population. Data for children remain sparse. Furthermore, at present, each condition is diagnosed with a separate test.

**Objectives:** The purpose of our study was to determine whether IDA,  $\alpha$ TT and  $\beta$ TT can be differentiated by a single easily measurable parameter, either well known or novel.

**Methods:** A retrospective case-comparative design was used. The study sample included 68 children with IDA,  $\alpha$ TT, or  $\beta$ TT, and a control group without any anemia at all, who attended the Hematology Clinic at Schneider Children's Medical Center of Israel from 2001 to 2007. In all cases, the diagnosis was based on gold standard methods, including genetic analysis for the  $\alpha$ -thalassemia mutation. Data on the initial blood workup (biochemistry, blood count, reticulocyte count) were recorded.

**Results:** The differences in specific clinical parameters identified in previous studies between adult patients with IDA or  $\beta$ TT were found to be true for children as well as adults. We did not find a significant difference between  $\beta$ TT and  $\alpha$ TT, and no differences have been documented in previous work. Original findings include: red blood cell count x reticulocyte-specific hemoglobin content significantly differentiated IDA from  $\alpha$ TT, and log-ferritin differentiated among IDA,  $\alpha$ TT, and  $\beta$ TT.

**Conclusions:** Our findings suggest parameters that can serve as easy and accessible clinical indices to determine the cause of anemia. Further data would make it possible to calculate threshold values and incorporate them in automatic laboratory instruments.

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### The protective effect of the immunomodulator AS101 against chemotherapy-induced female reproductive damage

M. Fishel Bartal, A. Carmely, Y. Kalechman, B. Sredni and D. Meirou

Department of Obstetrics and Gynecology, Sheba Medical Center, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

**Background:** For young cancer patients, the success of treatment using regimens that are toxic to gonadal function has made infertility an important problem. Cyclophosphamide (Cy) is extensively used as an anticancer drug, but it has significant detrimental effects on female fertility. The methods used today to overcome potential treatment-induced infertility have serious drawbacks and safety risks. The non-toxic immunomodulatory compound AS101 has been shown in diverse animal studies to have beneficial effects; moreover, clinical phase 2 trials on cancer patients treated with AS101 in combination with chemotherapy showed that treatment with AS101 induced a significant reduction in the severity of neutropenia, thrombocytopenia and alopecia that accompany chemotherapy.

**Objectives:** We investigated whether AS101 has a protective effect against the loss of primordial follicles induced by Cy.

**Methods:** In the experiment we used 6–8 week old Balb/c female mice. In the first experiment Cy was injected i.p. in different dosages: 0, 75 mg/kg, 100 mg/kg or 150 mg/kg. Every alternate day 10  $\mu$ g/mouse AS101 was injected twice before and three times after the Cy injection. In a second experiment, 75 mg/kg Cy was injected once a week for 4 weeks with or without concurrent AS101 injections three times a week. All mice were sacrificed 7 days after the last Cy injection. Ovaries were removed 7 days after cytotoxic treatment. Two examiners, who were unaware of the treatments, counted the number of ovarian primordial follicles (PMF).

**Results:** In the first experiment, administration of 75 mg/kg, 100 mg/kg, and 150 mg/kg of Cy reduced the number of ovarian PMF

to  $51\% \pm 15.1\%$ ,  $48 \pm 12.6\%$ , and  $38 \pm 22.2\%$  of the control group, respectively. In the groups treated with Cy+AS101 the PMF count was  $94.8 \pm 14.4\%$ ,  $99.8 \pm 16.7\%$ , and  $60.7 \pm 5.3\%$  of the control group, respectively. Significant protection was conferred by AS101 in all Cy doses ( $P < 0.01$ ). In the second experiment, administration of 75 mg/kg once a week for 4 weeks reduced the PMF count to  $12.2 \pm 9.5\%$  of the control group. In the group treated with Cy+AS101 the PMF count was  $43.0 \pm 12.9\%$  of the control group, which was significantly higher than in the Cy group ( $P < 0.01$ )

**Conclusions:** Our results show that treatment with the non-toxic compound AS101 can significantly protect against the reduction in the PMF pool induced by either single or multiple Cy doses. Further research is needed to elucidate the mechanisms by which AS101 confers ovarian chemoprotection.

## The effect of systemic administration of DHEA on persistent latent inhibition induced by mk-801

T. Levy, I. Weiner, R. Maayan and A. Weizman

Geha Medical Center and Felsenstein Research Center, affiliated to Department of Psychology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

**Background:** Dehydroepiandrosterone (DHEA) and DHEA-sulfate are steroid hormones that have an independent biological role in the brain, by acting on membranous ligand-gated ion channels [1]. Previous research suggests that these hormones have a positive modulatory effect on the NMDA receptor [2]. Studies examining the therapeutic effect of systemic administration of DHEA to schizophrenic patients indicated positive results, especially on the negative symptoms

of schizophrenia [3]. The latent inhibition (LI) model is a neuropsychological and cognitive model in humans and animals that demonstrates normal inhibited conditioning as a result of previous non-reinforced exposure to the conditioned stimulus. LI indexes the ability to ignore irrelevant stimuli. Central neural manipulations characteristic of schizophrenia cause aberrant LI and are considered to model the attention deficit exhibited in schizophrenic subjects. Antagonism of NMDA, which produces behavioral and cognitive deficits that closely mimic symptoms of schizophrenia, induces abnormal persistent LI in rodents, which is reversed by some atypical antipsychotic drugs [4].

**Objective:** The aim of this study was to examine indirectly the possible ameliorating effect of DHEA on schizophrenia-typical behavior abnormalities, by means of the LI procedure.

**Methods:** We examined the effect of DHEA (2 mg/kg, 5 mg/kg) on persistent LI induced by MK-801 (0.05 mg/kg).

**Results:** We found that DHEA reverses abnormal persistent LI induced by MK-801.

**Conclusions:** The results provide further validation to the hypothesis that DHEA has a therapeutic effect in schizophrenia, suggesting an NMDA-mediated mechanism. By confirming the hypothesis we provide a platform for examining the mechanism of action in animals and patients.

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### Capsule

#### Transcription factor Eos is selectively required for Foxp3-mediated gene suppression in T cells

CD4<sup>+</sup> regulatory T cells (T<sub>regs</sub>) are critical for keeping our immune system in check. They prevent immune responses from getting out of hand and keep autoimmunity at bay. By activating the expression of some genes and turning off expression of others, the master regulatory transcription factor of T<sub>regs</sub>, Foxp3, endows these cells with the appropriate gene expression program to mediate their suppressive effects. Pan et al. demonstrated that the transcription factor Eos is selectively required for Foxp3-mediated gene suppression in mice. Genes

normally suppressed by Foxp3 in T<sub>regs</sub> remained "on" when Eos expression was suppressed, whereas genes activated by Foxp3 were unaffected. T<sub>reg</sub> function was also affected by Eos suppression. With half their genetic program disrupted, these cells resembled an intermediate between T<sub>regs</sub> and conventional CD4<sup>+</sup> T cells – unable to suppress immune responses properly and partially responsive to T cell-activating stimulation.

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Eitan Israel

“Before you embark on a journey of revenge, dig two graves”

Confucius