

## Letters to the Editor

### Colchicine treatment in familial Mediterranean fever: An indirect effect on *in vitro* serum amyloid A secretion via leukocyte derived factors

Sirs,

Reactive amyloidosis is one of the most serious complications in many chronic inflammatory conditions such as inflammatory bowel disease, juvenile chronic arthritis and familial Mediterranean fever (FMF). Reactive amyloidosis is characterized by the extracellular deposit of insoluble fibrils containing various proteins, mainly amyloid A (1), which is structurally similar to, and immunologically cross-reactive with its precursor, the acute response protein, serum amyloid A (SAA) (2). SAA is produced and secreted by the liver, and its production and secretion are controlled by proinflammatory cytokines, mainly interleukin-1 (IL-1) and IL-6 acting in synergy (3, 4).

It is well recognized that continuous colchicine treatment prevents amyloidosis in FMF patients (5) and has successfully been used in the treatment of reactive amyloidosis in ulcerative colitis (6).

The objective of the present study was to examine whether colchicine exerts its effects on SAA synthesis via peripheral blood mononuclear cell (PBMC)-derived factors. Ten FMF patients, aged 3 to 14 years, who fulfilled the criteria of Pras (7) for the diagnosis of FMF, were studied before they initiated colchicine regimen and again during treatment after it had been in progress for at least one month.

Blood was obtained from each patient after receiving informed consent. The PBMC were separated on Ficoll density gradients, washed, and cultured at  $5 \times 10^6$  cells/ml in RPMI 1640 medium, with and without 10 g/ml of lipopolysaccharide (LPS). After 48 hrs the cell-free culture medium (CM) was collected.

Human hepatoma Hep3B cells were seeded at 50,000 cells/ml, in RPMI 1640 medium

with 10% fetal calf serum, after five days the PBMC-derived CM was added and cultured for 24 hours. Levels of secreted SAA in the Hep3B-derived cell-free culture medium were determined in duplicates by a modified ELISA inhibition assay (8).

SAA secreted by hepatoma cells exposed to untreated PBMC culture medium from non-treated patients was  $1.8 \pm 3.2$  g/ml while a higher mean level ( $6.2 \pm 6.5$  g/ml) but statistically not significant  $p = 0.07$ , was obtained from these patients during treatment with colchicine. Addition of LPS to PBMC from untreated patients resulted in the induction of a significantly ( $p = 0.02$ ) higher mean level of SAA ( $4.7 \pm 5.3$  g/ml) (Fig. 1). However, as opposed to the increased SAA levels induced in hepatoma cells by CM from LPS-stimulated PBMC of untreated patients, the SAA level obtained in response to CM of LPS-stimulated PBMC from colchicine-treated patients was significantly reduced, from  $6.2 \pm 6.5$  g/ml to  $3.8 \pm 4.4$  g/ml ( $p = 0.04$ ). The results of this study indicate, in agreement with previous reports, that the secretion of SAA from hepatoma cells may be stimulated by factors released from activated mononuclear cells, probably including proinflammatory cytokines, *e.g.* IL-6, IL-1 and TNF (9).

Surprisingly, when LPS was added to the mononuclear cells from colchicine-treated individuals, the quantity of SAA obtained from the hepatoma cultures decreased markedly. This may result from a differential cytokine response induced by the combination of colchicine and LPS. Since colchicine was previously shown to inhibit the LPS induced TNF secretion by blocking the transcription of the gene (10), it is possible that the lingering effects of the exposure to colchicine might have blocked the effects of LPS in the present study.

This effect of colchicine is probably independent of its anti-inflammatory properties as implied from the finding that colchicine prevents amyloidosis in FMF patients even if they continue to have inflammatory at-

tacks (4).

If indeed colchicine prevents SAA secretion by blocking the effect of LPS and possibly of other stimuli, its wider use in preventing reactive amyloidosis should be considered.

J. BARASH<sup>1</sup>, MD

A. PIROGOVSKI<sup>1</sup>, MD

A. LIVNEH<sup>2</sup>, MD

N. BREZNIAK<sup>2</sup>, MD, DMD

Y. DROR<sup>1</sup>, MD

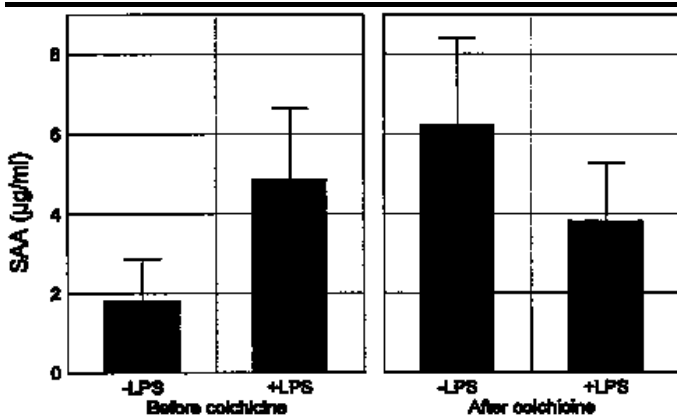
T. HAHN<sup>1</sup>, PhD

*The Pediatric Division and Pediatric Research Laboratory, Kaplan Medical Center, Rehovot, and Heller Institute of Medical Research, Sheba Medical Center, Tel-Hashomer, Israel.*

*Please address correspondence to: Dr. J. Barash, Director, Pediatric Day Care, Kaplan Hospital, POB 1, Rehovot 76100, Israel. e-mail: barashj@inter.net.il*

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**Fig. 1.** The effect of culture medium from monocytes from 10 FMF patients before and during colchicine treatment, with or without LPS stimulation, on SAA production by Hep3B cells (mean  $\pm$  SEM).