Letter to the Editor

The polyamine spermine promotes survival and activation of human eosinophils

To the Editor:

Allergic rhinitis and most phenotypes of asthma involve accumulation of eosinophils in the airways. The cytokines GM-CSF, IL-5, and IL-3 enhance eosinophil survival and contribute to initiation and maintenance of eosinophilic airway inflammation in asthmatic patients. However, their neutralization in clinical trials only partially depleted airway eosinophilia, suggesting the existence of other relevant life-supporting factors.[1] Spermine, spermidine, and putrescine are ubiquitous cationic polyamines with basic functions related to nucleic acid packaging, DNA replication, apoptosis, transcription, and translation. Intracellular levels of polyamines are regulated by biosynthesis, catabolism, cellular uptake, and efflux. Polyamine synthesis is initiated by arginase, which converts arginine to ornithine. Putrescine is synthesized from ornithine and further converted to spermidine and spermine.[2-4] Interestingly, arginase activity and polyamine levels are increased in both serum and sputum from asthmatic patients and in experimental models of asthma, suggesting a role in allergic airway diseases.[4-6] Hence we aimed to investigate whether polyamines directly regulate human eosinophil survival and activation, focusing on spermine, the most active polyamine.

Eosinophils were isolated from venous blood of eosinophilic donors. For donors' characteristics, see Table E1 in this article's Online Repository at www.jacionline.org, and for the materials

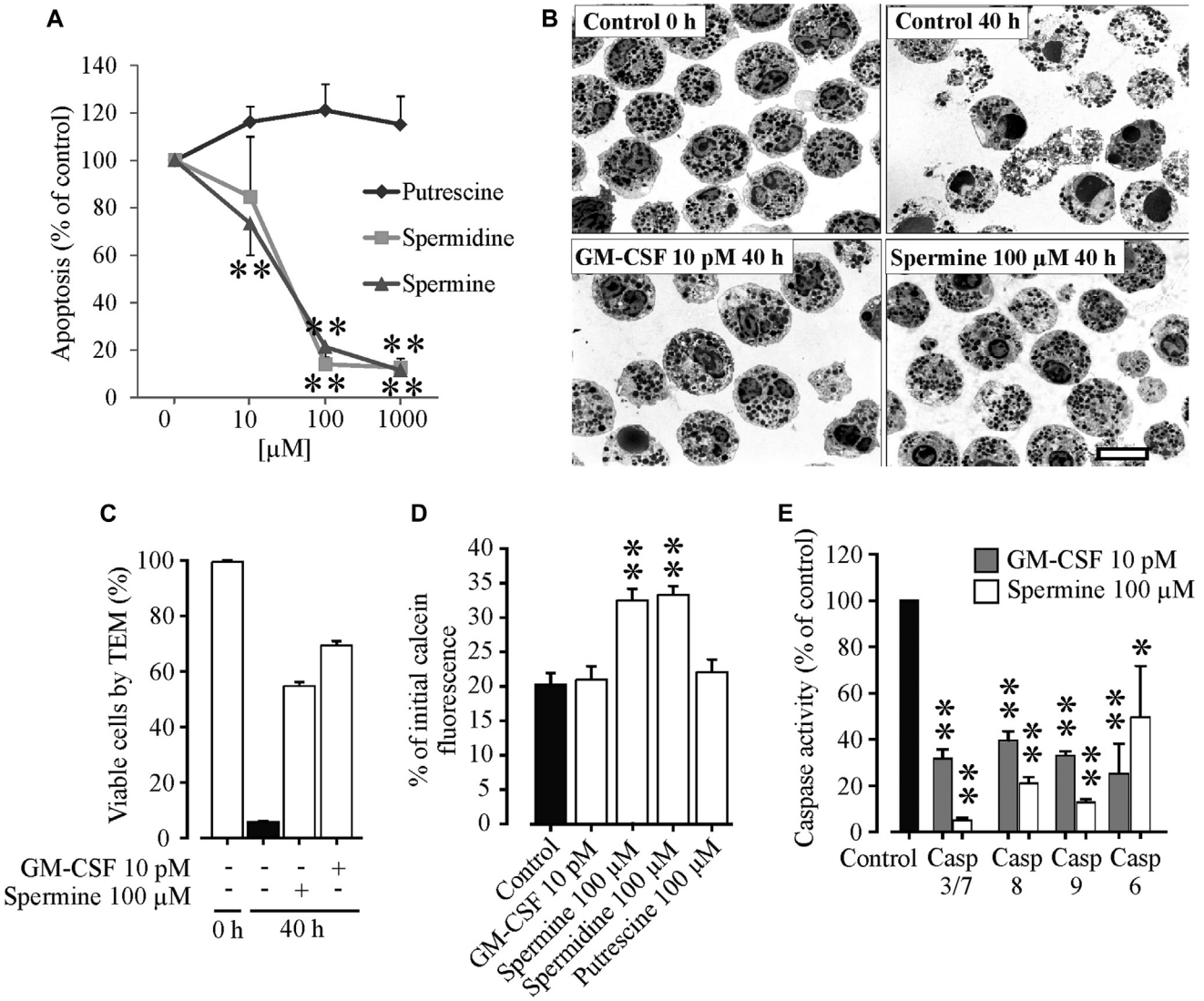

FIG 1. Spermine prolongs eosinophil survival by inhibiting mPT and caspase activity. Incubation times were 40 hours **(A)**, 18 hours **(D)**, and 16 hours (caspases 3/7, 8, and 9) or 40 hours (caspase 6; **E**). **B,** Representative figures from transmission electron microscopic analysis are shown. In Fig 1, *D*, mPT was determined based on calcein/$CoCl_2$ staining. Shown are means ± SEMs of 4 to 6 (Fig 1, *A* and *D-E*) and 2 **(C)** experiments, respectively. $*P < .05$ and $**P < .01$ versus untreated controls.

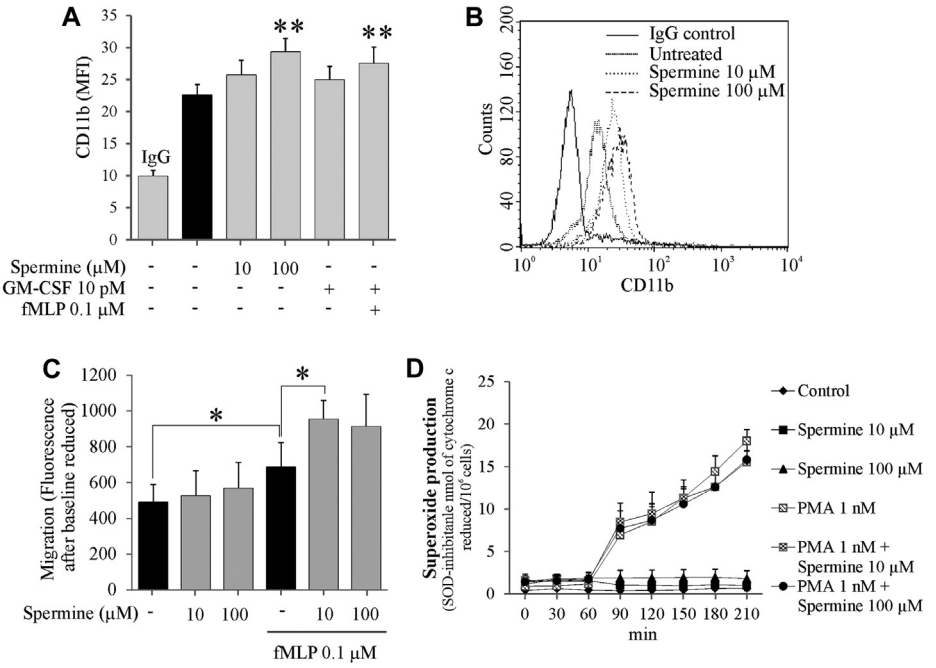

FIG 2. Spermine enhances eosinophil activation. Incubation times were 16 hours (**A** and **B**; fMLP added at the end for 15 minutes) and 2 hours **(C)**. In Fig 2, *B*, representative overlaid plots of spermine effect in eosinophils from an asthmatic patient are shown. **D,** Phorbol 12-myristate 13-acetate *(PMA)* was added at the 1-hour time point. Shown are means ± SEMs of 6 to 8 experiments. $*P < .05$ and $**P < .01$ versus control (ie, *black bar*, Fig 2, *A* and *C*).

and methods used in this study, see the Methods section in this article's Online Repository at www.jacionline.org.

Spermine and spermidine, but not putrescine, prevented apoptosis-related DNA fragmentation in human eosinophils (Fig 1, *A*). For comparison, the well-known eosinophil survival–prolonging cytokine GM-CSF (10 pmol/L) decreased the number of apoptotic cells to 36.5% ± 7.4% of control values ($n = 5$, $P < .05$). Spermine, with the highest number of positive charges, was the most potent polyamine, with a median effective concentration of 15.2 ± 3.0 μmol/L (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org), and was used in further studies.

Analysis of eosinophil morphology by means of bright-field microscopy further suggested that spermine promotes eosinophil survival (see Fig E1, *B*). This observation was confirmed by means of transmission electron microscopic analysis, which clearly showed that both spermine and GM-CSF inhibit apoptosis and increase the number of viable cells (Fig 1, *B* and *C*). Spermine did not cause primary necrosis, as shown in Table E2 in this article's Online Repository at www.jacionline.org. Our preliminary experiments also suggested that spermine inhibits apoptosis in eosinophils both from healthy donors and asthmatic patients (see Fig E2 in this article's Online Repository at www.jacionline.org).

The survival-promoting effects of spermine (median effective concentration = 15 μmol/L in the present study) are likely to be pathophysiologically relevant because levels of up to 30 μmol/L spermine were found in the blood of asthmatic patients.[5] Interestingly, studies in mice suggest that the concentration of spermine is higher in the lungs compared with the systemic circulation (lung homogenate, 316 nmol/g [nanomoles per gram ≈ micromoles per liter]; blood, 4 μmol/L).[7] The present study is the first to show that polyamines inhibit eosinophil apoptosis. However, their effect on cell death might depend on the cell type[2] because spermidine was reported to enhance survival of human PBMCs, and that effect was due to reduced necrosis rather than inhibited apoptosis.[8]

Next, we continued by elucidating the antiapoptotic mechanism of spermine. Inhibitors of the kinases Janus kinase 2 (JAK2), phosphoinositide 3-kinase (PI3K), extracellular signal-regulated kinase (ERK), and p38 and the transcription factor nuclear factor κB (NF-kB) did not alter the survival-promoting effect of spermine (see Tables E3-E5 in this article's Online Repository at www.jacionline.org), suggesting that spermine acts through a different mechanism when compared with the survival-prolonging cytokines GM-CSF and IL-5.[1] Spermidine has previously been shown to mediate survival of human blood mononuclear cells by inducing autophagy,[8] but spermine had no such effect in human eosinophils in the present study (see Fig E3 in this article's Online Repository at www.jacionline.org).

Mitochondrial permeability transition (mPT) is a mechanism mediating the intrinsic form of apoptosis and found to be involved in spontaneous eosinophil apoptosis.[9] The mitochondrial membrane has been reported to contain 2 binding sites for spermine.[2] Furthermore, spermine and spermidine, but not putrescine, prevented mPT in isolated mitochondria.[2] Therefore we tested whether polyamines inhibit mPT in human eosinophils using a method based on calcein acetoxymethyl ester (AM) and $CoCl_2$.[9] Calcein AM fluorescence is quenched by $CoCl_2$, which only enters mitochondria with ongoing mPT. Treatment with spermine or spermidine (100 μmol/L) clearly increased calcein AM fluorescence in the presence of $CoCl_2$ when compared with that seen in untreated eosinophils or cells treated with putrescine or GM-CSF (Fig 1, *D*, and see Fig E4 in this article's Online