Brief communication

Corticosteroids depress IFN-α–producing plasmacytoid dendritic cells in human blood

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Glucocorticoids are strongly immunosuppressive and are associated with reactivation of some intracellular infections. The plasmacytoid dendritic cell is a rare blood mononuclear cell detected through its production of IFN-α in response to herpes simplex virus and by surface immunophenotyping. We here report that steroid administration results in a decrease of IFN-α generation of approximately 25-fold, accompanied by reduction in circulating plasmacytoid dendritic cell numbers. Both parameters return to normal within days after steroid cessation. (J Allergy Clin Immunol 2001;108:446-8.)

Key words: *IFN-producing cells, dendritic cells, plasmacytoid, corticosteroids, steroid effects, pDC, immunosuppression, T_H1 immunity, flow cytometry*

Plasmacytoid dendritic cells (pDCs) in human blood, detected by their rapid, copious production of IFN-α in response to enveloped viruses, have been implicated in AIDS pathogenesis and resistance to intracellular opportunistic organisms.[1,2] After viral stimulation, IFN production by pDCs fosters T_H1 differentiation of immature T cells as the pDCs mature into type 2 dendritic cells.[3-5] Recent studies have investigated the effects of corticosteroids on type 1 dendritic cells.[6-7] Effects on pDCs of corticosteroids, substances known to be anti-inflammatory and immunosuppressive,[8,9] have not been investigated.

We analyzed pDC function (as IFN-α generation) in a case control study of patients treated with steroids for various indications and in serial studies of patients and healthy volunteers. We also enumerated circulating pDCs by flow cytometry in the studies on normal subjects and determined the in vitro effect of steroids on IFN-α generation.

Abbreviation used
pDC: Plasmacytoid dendritic cell

MATERIAL AND METHODS

Study subjects

Studies were approved by the institutional review boards at Long Island Jewish Medical Center and Saint Vincents Hospital and Medical Center. PBMCs were collected[1,2] from 26 controls and 29 of 30 patients being treated with steroids for a range of disorders. One of the 30 patients was studied only just after stopping steroids. Fourteen of the patients had been on chronic daily steroids (>100 days), and 15 had been treated for 1 to 60 days at the time of study. Five patients were followed after discontinuation of steroids.

PBMCs from 4 healthy volunteers were studied before, during, and after 4 days of 30-mg-per-day oral prednisone (Mutual Pharmaceuticals, Philadelphia, Pa).

IFN generation and bioassay

Ficoll/Hypaque-isolated PBMCs were cultured overnight with UV light–inactivated herpes simplex virus (provided by Patricia Fitzgerald-Bocarsly, PhD, New Jersey College of Medicine), as previously described.[1,2] IFN bioassay was done in 2 ways: (1) protection of human foreskin fibroblasts from cytopathic effects after infection by vesicular stomatitis virus as compared in parallel with the protective effects of serially diluted IFN standards; (2) indicator cells containing structural luciferase genes downstream from type I IFN response elements (PIL cells) constructed for quantitative detection of IFN by Michael G. Tovey, MD (Laboratory of Viral Oncology, Villejuif, France). Luciferase generation is linearly proportional to IFN concentration over a 20-fold range and is sensitive to 0.05 units of IFN per milliliter. The 2 assays gave equivalent results in 20 randomly chosen supernatants that were assayed by each method. Carryover herpes simplex virus did not elicit IFN production by either indicator cell.

Flow cytometry

pDCs were defined as positive for CD4 and CD123 but negative for CD11c (FITC-conjugated, Coulter, Inc., Hialeah, Fla) and lineage markers (FITC-conjugated CD3, CD14, CD16, CD19, CD20, and CD56, BDIS, San Jose, Calif [kindly supplied by Noel Warner, PhD, BDIS, San Jose Calif]).[3-5] A total of 80,000 cells were enumerated for each run (FACScalibur, BDIS).

Statistical methods

The Student 2-tailed *t* test for independent samples was used, as was the Bonferroni correction for multiple comparisons (SPSS for Windows, Release 5.0, SPSS Inc, Chicago, Ill).

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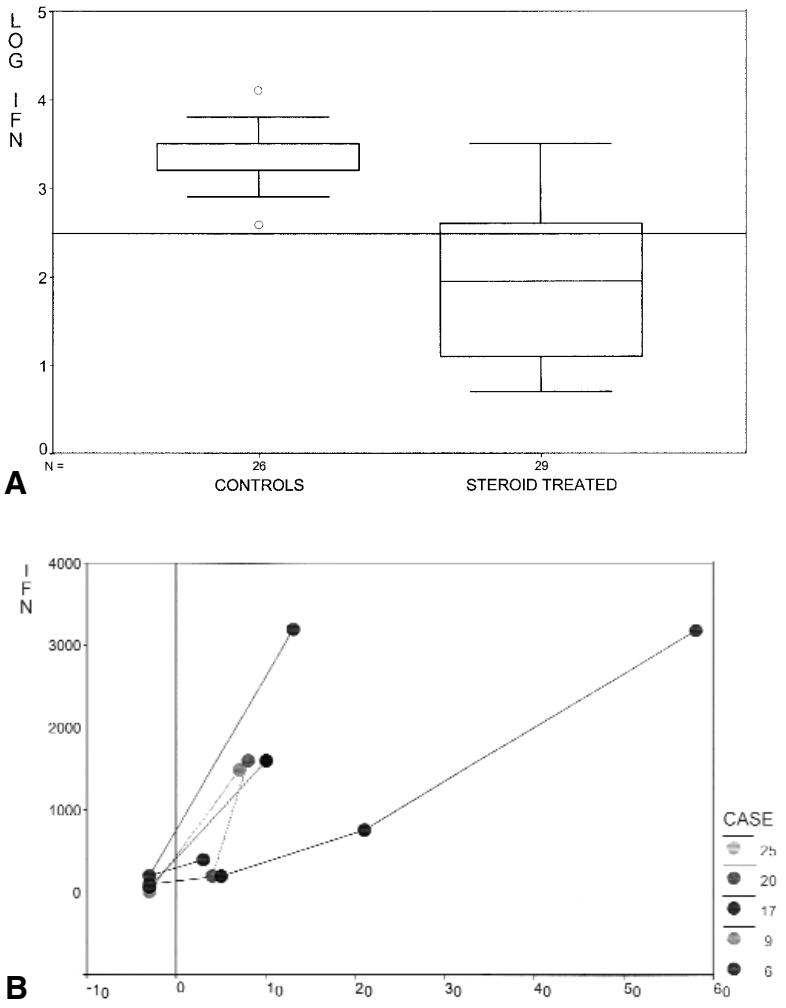


FIG 1. A, Corticosteroid effect on IFN-α generation. *Ordinate:* $\log_{10}$ IUs of IFN per milliliter. *Left boxplot,* PBMCs of normal controls (n = 26) produced 3.226 ± 0.329 $\log_{10}$ IU/mL (mean ± 1 SD). *Right boxplot,* PBMCs from subjects receiving corticosteroid therapy (n = 29) produced 2.077 ± 0.707 $\log_{10}$ IU/mL ($P < .0005$). **B,** Recovery of IFN-α generation after cessation of corticosteroids. Five patients who were on steroids were followed after discontinuation of the drugs. IFN-α generation during treatment, shown to the left of the zero reference line, reflects the geometric mean of IFN-α generation for all determinations in each of 4 cases (94 ± 80 IU/mL [mean ± 1 SD]). To the right of zero, points are for 5 individuals (case 20 was studied during 2 recovery periods) at the days indicated. *Ordinate:* IFN-α (IU/mL); *Abscissa:* days following steroids (0 = last day on steroids). IFN-α generation after steroids were stopped rose significantly ($P = .01$ to .03, depending on the days analyzed).

RESULTS AND DISCUSSION

PBMCs of patients receiving steroids (Fig 1, *A*) generated 1.9277 ± 0.799 $\log_{10}$ IU of IFN per milliliter (geometric mean 85 IU/mL), approximately 25-fold below the average for normal controls (3.319 ± 0.282 $\log_{10}$ IU of IFN per milliliter, or approximately 2100 IU/mL; $P < .0005$). Those available for study after steroids were stopped showed rapid recovery of IFN generation (Fig 1, *B*).

Four normal volunteers were followed through a 4-day course of oral prednisone (30 mg/day). By day 4 of steroids, the geometric mean IFN-α generation fell to 160 IU/mL ($\log_{10}$ mean ± SEM, 2.196 ± 0.486) from a presteroid geometric mean of 3000 IU/mL (3.46 ± 0.326; $P = .0042$). After steroids were stopped, IFN production increased, normal levels being achieved by day 3. Recovery curves closely resembled those seen in the patient study (Fig 1, *B*). In the same 4 volunteers, the mean (± 1 SD) number of circulating pDCs was 3.58 ± 1.44/mm^3 before steroids; it dropped to 1.05 ± 1.18/mm^3 after 3 to 4 days of steroids ($P = .032$). pDC numbers also returned to pretreatment levels by 2 to 3 days after cessation of steroids. Circulating pDC numbers were proportionally more affected by steroid therapy than were numbers of circulating granulocytes and lymphocytes.

A physiologic reciprocal relationship between endogenous cortisol levels and IFN-α production has also recently been reported.[10]

Exposure of normal PBMCs to hydrocortisone or 6α-methylprednisolone in a 5-6 log dilution range, shown to have effects in other systems, did not directly affect the production of IFN-α in 4 experiments (data not shown).

Modulation of the immune response by steroids is complex; it is already known to include effects on certain other subclasses of dendritic cells.[5-7] Depression of pDCs by corticosteroids likely contributes to the clinical immunosuppression observed with this treatment modality.

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REFERENCES

1. Siegal FP, Lopez C, Fitzgerald PA, Shah K, Baron P, Leiderman IZ, et al. Opportunistic infections in acquired immune deficiency syndrome result from synergistic defects of both the natural and adaptive components of cellular immunity. J Clin Invest 1986;78:115-23.
2. Siegal FP, Fitzgerald-Bocarsly P, Holland BK, Shodell M. Interferon-α generation and immune reconstitution during antiretroviral therapy for human immunodeficiency virus infection. AIDS. In press.
3. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, et al. Identity of the principal type-I IFN-producing cells in human blood. Science 1999;284:1835-37.
4. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nature Med 1999;5:919-23.
5. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent Th1 polarization. Nat Immunol 2000;1:305-10.
6. Matyszak M, Citterio S, Rescigno M, Castagnoli P. Differential effects of corticosteroids during different stages of dendritic cell maturation. Eur J Immunol 2000;30:1233-42.
7. Piemonti L, Monti P, Allavena P, Sironi M, Soldini L, Leone, BE, et al. Glucocorticoids affect human dendritic cell differentiation and maturation. J Immunol 1999:162:6473-81.
8. Didonato JA, Saatcioglu F, Karin M. Molecular mechanisms of immunosuppression and anti-inflammatory activities by glucocorticoids. Am J Respir Crit Care Med 1996;154(2 Pt 2):S11-S15.
9. Franchimont D, Galon J, Gadina M, Visconti R, Zhou Y, Aringer M, et al. Inhibition of Th1 immune response by glucocorticoids: dexamethasone selectively inhibits IL-12-induced Stat4 phosphorylation in T lymphocytes. J Immunol. 2000;164:1768-74.
10. Reissland P, Wandinger KP. Increased cortisol levels in human umbilical cord blood inhibit interferon alpha production of neonate. Immunobiology 1999;200:227-33.