1257

EFFECTS ON OTHER LEUKOCYTES

Evaluation of the Influence of FK 506, Rapamycin, and Cyclosporine on Processing and Presentation of Particulate Antigen by Macrophages: Assessment of a Drug "Carry-Over" Effect

M.H. Cooper, S.H. Gregory, A.W. Thomson, J.J. Fung, T.E. Starzl, and E.J. Wing

FK 506 is 10 to 100 times more potent than cyclosporine (CyA) in inhibiting T-cell responses to mitogenic and allostimulatory signals in vitro.[1] Both drugs affect the early events subsequent to antigen–T-cell receptor interaction that characterizes T-cell activation.[1] In contrast, rapamycin, which is structurally similar to FK 506, inhibits cytokine-mediated, but not T-cell receptor-mediated activation.[1] The processing and presentation of antigen by macrophages is integral to the early events of T-cell activation. A recent report indicates that CyA interferes with these macrophage functions.[2] In view of this observation, we examined the effects of FK 506 and rapamycin on the processing and presentation of particulate antigen using a model composed of bone marrow-derived macrophages, heat-killed *Listeria monocytogenes* (HKLM), and a listerial-reactive CD4+ T-cell clone.

MATERIALS AND METHODS

Animals

Eight- to 12-week-old female C57BL/6J mice purchased from Jackson Laboratories, Bar Harbor, Me, were used in all experiments. The animals were housed in accordance with the guidelines proposed by the Institute of Laboratory Animals Resources, National Research Council.

Bone Marrow-Derived Macrophages

Murine bone marrow-derived macrophages were generated in the presence of L-cell–conditioned medium as previously reported.[3] Cells harvested from day 11 cultures were used for the experiments described.

Bacteria

The culture and maintenance of *L monocytogenes* (EGD strain) have been reported previously.[4] HKLM was prepared by incubating bacterial suspensions for 1 hour at 60°C. HKLM was washed twice, suspended in saline at 1×10^9 cells/mL, and frozen at −80°C until required.

CD4+ T-Cell Clone

The production and characterization of the *Listeria*-specific T-cell clone 5A9 have been previously described.[5] Viable cells were purified by Ficoll-Hypaque centrifugation prior to use.

Immunosuppressants

FK 506 was a gift from Fujisawa Pharmaceutical Company Ltd, Osaka, Japan. Rapamycin was a gift from Wyeth-Ayerst Laboratories, Princeton, NJ. CyA was purchased from Sandoz Pharmaceuticals, Basel, Switzerland. All drugs were obtained in pure powder form. Ten millimolar stock solutions were prepared in absolute ethanol. All subsequent dilutions were made in tissue culture medium (RPMI-1640).

Experimental Assay of Antigen Processing and Presentation

Bone marrow-derived macrophages (3×10^4 cells/well) were seeded into 96-well microtiter plates and incubated for 2 hours at 37°C in order to allow the cells to attach. FK 506, rapamycin, or CyA was added and the cells were incubated for an additional 2 hours. The cell monolayer was then washed four times according to a protocol considered adequate for the removal of CyA.[6] One million HKLM and 3×10^5 5A9 T lymphocytes suspended in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 1 mmol/L sodium pyruvate, 1 mmol/L L-glutamine, 1% essential and nonessential amino acids (M.A. Bioproducts, Walkerville, Md), 5×10^{-5} M 2-mercaptoethanol, and 1% penicillin/streptomycin/fungizone (Gibco, Grand Island, NY) were added to each well and the cells were cocultured for 3 days. To assess T-cell proliferation, each well received 1 μCi of tritiated thymidine during the last 18 hours of the culture period and the counts incorporated were determined by liquid scintillation counting.

Concanavalin A Stimulation of Normal Splenocytes

Normal mouse splenocytes (1×10^5 cells per well) and 2 μg/mL of concanavalin A (ConA) (Sigma Chemicals, St Louis, Mo) were added to cultures of drug-treated macrophages. Splenocyte proliferation was assessed by tritiated thymidine incorporation on day 3.

From the Departments of Surgery (M.H.C., A.W.T., J.J.F., T.E.S.) and Medicine (S.H.G., E.J.W.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Supported by Grants DK 29961 (to T.E.S.) and AI 24141 (to E.J.W.), National Institutes of Health, Bethesda, Maryland.

Address reprint requests to Mark H. Cooper BSc, MB ChB, Department of Surgery, Division of Transplantation, University of Pittsburgh, 15th Floor Biomedical Science Tower, Pittsburgh, PA 15232.

© 1991 by Appleton & Lange

0041-1345/91/$3.00/+0

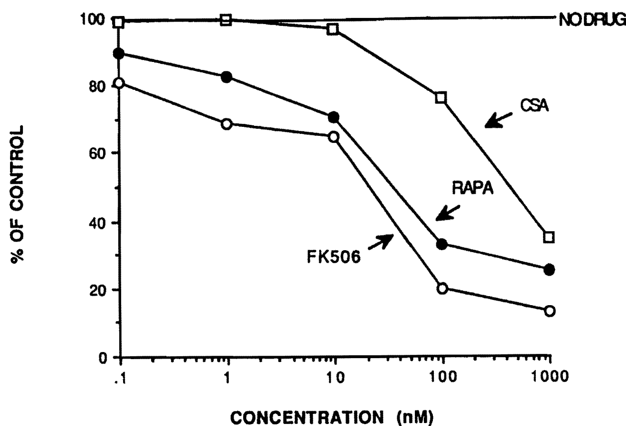

Fig 1. Antigen-specific T-cell proliferation is suppressed in cocultures containing macrophages pretreated with FK 506, CyA, or rapamycin. 5A9 T lymphocytes and HKLM were cocultured for 3 days with bone marrow-derived macrophages pretreated with drug at the concentrations indicated. Cell proliferation was assessed by tritiated thymidine incorporation during the last 18 hours of the culture period. 5A9 T cells cocultured with HKLM and untreated macrophages incorporated 12,826 cpm. Data are the mean percentages of this control value, calculated from three identical wells.

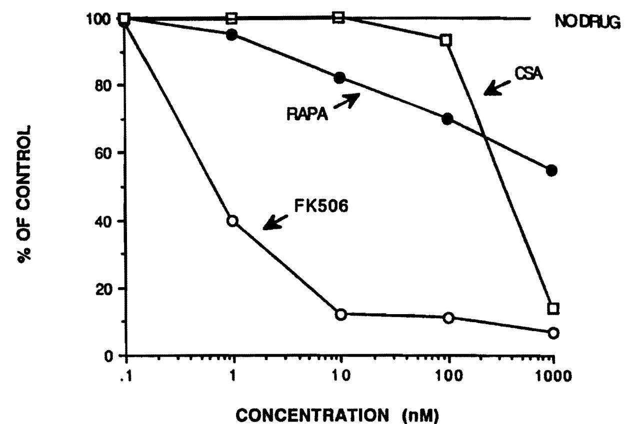

Fig 2. ConA response of normal splenocytes is suppressed in cocultures containing drug-pretreated macrophages. Normal splenocytes and ConA (2 μg/mL) were cocultured with bone marrow-derived macrophages pretreated with drug at the concentrations indicated. Cell proliferation was assessed by tritiated thymidine incorporation during the last 18 hours of the culture period. ConA-stimulated splenocytes cocultured with untreated macrophages incorporated 16,325 cpm. All values are the mean percentages of this control value calculated from three identical wells.

RESULTS

FK 506, CyA, or rapamycin-pretreated macrophages suppress the proliferative response of 5A9 T cells to HKLM. The proliferative response of 5A9 T cells to HKLM was significantly reduced when the cells were cocultured with macrophages pretreated with drug (Fig 1). This inhibitory effect was dose-dependent. FK 506 was more potent than rapamycin which, in turn, was more potent than CyA.

FK 506, CyA, and rapamycin-pretreated macrophages suppress the blastogenic response of normal splenocytes to ConA. Studies were undertaken to determine whether the inhibitory effects of these drugs on T-cell proliferation were due to drug carry over rather than on the capacity of macrophages to process and/or present antigen. Macrophages were incubated with various concentrations of each drug as above. The cell monolayer was then washed four times, and normal splenocytes and ConA (a stimulant that induces T-cell proliferation independently of our drug-treated macrophages of interest) were then added. T-cell proliferation was measured as previously. A significant suppression of the T-cell response to ConA was seen when cells were cocultured with macrophages pretreated with drug (Fig 2). This effect was much more pronounced with FK 506 than with rapamycin. In the case of CyA, appreciable impairment was only found in cocultures containing macrophages preexposed to 1,000 nm. These data demonstrate that the diminished proliferative response of 5A9 T lymphocytes to HKLM observed in cocultures containing macrophages pretreated with drug was mainly due to drug carry over rather than the effect of the drug on antigen processing and/or presentation.

DISCUSSION

The incubation of HKLM and 5A9 T cells with drug-pretreated, macrophages resulted in significant suppression of antigen-induced T-cell proliferation. This suppression occurred regardless of whether macrophages were treated with FK 506, CyA, or rapamycin. The nonspecific proliferation of splenocytes to ConA was also inhibited in cocultures containing drug-treated macrophages indicating our inability to wash drug from out of our system.

Our results confirm previous studies by others in which a significant suppression of the T-cell response to antigen was observed when the cells were cocultured with macrophages pretreated with CyA.[2] These latter studies, however, gave little credence to the possibility of drug carry over.

We are currently exploring alternative methods of washing drug-treated macrophages, in an effort to eliminate the direct effect of drug carry over on T-cell function. The development of such methodology would then enable us to assess the effects of immunosuppressants on the ability of macrophages to process or present antigen to T cells.

REFERENCES

1. Dumont FJ, Staruch MJ, Koprak SL, et al: J Immunol 144:251, 1990
2. Palay DA, Cluff CW, Wentworth PA, et al: J Immunol 136:4348, 1986
3. Gregory SH: J Leukoc Biol 43:67, 1988
4. Wing EJ, Waheed A, Shadduck RK: Infect Immun 45:180, 1984
5. Magee DM, Wing EJ: J Immunol 141:3203, 1988
6. Hess AD, Tutschka PJ, Santos GW: J Immunol 126:961, 1981