The *in vivo* Cardiotoxic Effect of Eosinophilic Cationic Protein in an Animal Preparation

CHIHARU KISHIMOTO, M.D., CHRISTOPHER J.F. SPRY, M.D.*, PO-CHUN TAI, M.D.*, NOBUYOSHI TOMIOKA, M.D., AND CHUICHI KAWAI, M.D.

We studied the *in vivo* effects of eosinophilic cationic protein (ECP) on DBA/2 mice, and compared the cardiac lesions caused by ECP with those caused by encephalomyocarditis (EMC) virus. ECP caused myocarditis in two of five mice (40%), and EMC virus did so in five of five mice (100%). Cardiac lesions of ECP were mild and limited to the right ventricular wall, which differed from those in the mice with EMC virus inoculation, where both the right and left ventricles were involved. This experiment is the first demonstration of the *in vivo* cardiotoxicity of ECP.

The hypereosinophilic syndrome is a poorly understood disorder. Many organs are characteristically involved and cardiac lesions are very similar to those found in Löeffler's endocarditis.

Several substances have chemotactic activity for eosinophils, such as histamine, some antigen-antibody complexes and some components of human complement. Recently, Spry and Tai showed that a proportion of the circulating eosinophils in patients with hypereosinophilic syndrome possessed receptors for rabbit immunoglobulin (Ig) G-coated erythrocytes and actively phagocytosed lymphocytes coated with rabbit IgG or human C3b. These abnormal eosinophils, therefore, had characteristics of stimulated eosinophils with the capacity of responding to soluble substances in the blood. This eosinophilic material, eosinophilic cationic protein (ECP), may itself be responsible for cardiac injury. Thus, much attention has been paid to the role of ECP in *in vivo* cardiac damage in such patients.

We have already shown that the myocardio-trophic (M) variant of encephalomyocarditis (EMC) caused severe cardiac lesions in DBA/2 mice. We had an opportunity to examine the effects of ECP. The present experiment was designed to evaluate the *in vivo* effects of ECP in an animal preparation.

MATERIALS AND METHODS

Four-week-old DBA/2 mice were used.

Purified eosinophilic cationic protein, ECP, which was supplied by Drs. Christopher J.F. Spry and Po-Chun Tai, was diluted with saline at concentrations of 360 μg (protein)/0.5 ml (low ECP dose) and 900 μg/0.5 ml (high ECP dose), respectively.

The M variant of EMC virus was prepared as described previously.

The mice were injected intraperitoneally with ECP at different dosages (360 μg = low ECP group, n = 5 and 900 μg = high ECP group, n = 5).

Key Words:
- Hypereosinophilic syndrome
- Eosinophilic cationic protein
- DBA/2 mice
- Cardiotoxicity

*The Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan; Department of Immunology, Royal Postgraduate Medical School, London, England

This work was supported in part by a Research Grant for the study of Intractable Diseases from the Ministry of Health and Welfare of Japan

Mailing address: Chuichi Kawai, M.D., The Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, 54 Kawaracho Shogoin, Sakyo-ku, Kyoto 606, Japan

*Current address: Department of Immunology, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 ORE

1264 Japanese Circulation Journal Vol. 50, December 1986
with EMC virus containing $10^{2.5}$ 50% tissue culture infective dose (EMC virus group, n = 5) and with saline (control group, n = 5). These mice were observed daily for days after the treatment, and all surviving mice were sacrificed on day 7.

The hearts were sectioned longitudinally through four chambers, one half of the hearts were processed for pathologic study, and the other for virologic study.

RESULTS
One animal in the of high ECP group died on day 6 after the virus inoculation, but otherwise, other mice in each group survived until day 7.

There were two mice with myocarditis (40%) in the high ECP group, and five with myocarditis (100%) in EMC group. There were none with myocarditis (0%) in low EMC or control group.

As reported previously, extensive myocardial necrosis with calcification and cellular infiltrations (Fig. 1) was evident in the ventricles as well as atria in EMC group of mice. However, two mice with myocarditis in high ECP group demonstrated focal and mild myocardial necrosis with cellular infiltrations only in the right ventricle (Figs. 2, 3). This was apparently different from so-called spontaneous calcification seen in the right ventricular free wall in control group of
DBA/2 mice (Fig. 4).

Virus was isolated from the hearts of all five mice in EMC group. No viruses were isolated in high ECP, low ECP or control group.

DISCUSSION

As described previously\(^5,6\) the role of ECP, which is a purified extract from abnormal eosinophils in patients with hypereosinophilic syndrome\(^6\) has been significantly noted in the cardiac injury in such patients. We had an opportunity to investigate the effects of ECP; using established murine myocarditis models\(^7,8\). The low incidence of myocardial lesions in DBA/2 mice caused by human ECP might be due to the difference of species between mice used in this experiment and man\(^9\). The *in vitro* toxic effects of human ECP against rat heart mitochondria has already been reported\(^6\).

Although the incidence of myocarditis of ECP was low, this study clearly demonstrated the *in vivo* cardio-toxicity of ECP in an animal preparation.
REFERENCES

8. KISHIMOTO C, MATSUMORI A, OHMAE M, TOMIOKA N, KAWAI C: Electrocardiographic findings in experimental myocarditis in DBA/2 mice: Complete atrioventricular block in the acute stage, low voltage of the QRS complex in the subacute stage and arrhythmias in the chronic stage. *J Am Coll Cardiol* 3: 1461, 1984
10. SPRY CJF, TAI PC (personal communication).

*Japanese Circulation Journal* Vol. 50, December 1986