Abstract

Previous reports showed that cardiac surgery with cardiopulmonary bypass (CPB) impair cell-mediated immunity by using antigen-non-specific responses. This study elucidated the effects of cardiac surgery with CPB on antigen-specific immunity. Twenty patients who underwent elective cardiac surgery using CPB were randomly divided into two groups: group A (n = 10) and group B (n = 10) with and without steroid administration, respectively. Group C patients underwent off-pump CABG (n = 8). Peripheral blood mononuclear cells (PBMCs) were taken before and after surgery. Proliferation responses to pure protein derivative antigen were measured. The effects of CPB and steroid on T cell response and antigen-presentation were assessed by cross-stimulation between the preoperative and the postoperative PBMCs. Antigen-specific T cell responses decreased to about 5% of the preoperative values immediately after surgery with CPB, regardless of steroid administration. The T cell response in group B on POD 7 was significantly higher than that in group A. CPB impaired mainly T cell responses, and steroid administration enhanced impairment of T cell response and antigen-presentation. Open-heart surgery with CPB severely impaired antigen-specific immunity. Steroid administration enhanced the impairment of antigen-presentation as well as T cell function, and retarded the recovery of antigen-specific immunity.

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1. Introduction

Several investigators have reported that cell-mediated immunity is impaired by cardiac operations performed with cardiopulmonary bypass (CPB) [1–9]. The indicators of cell mediated immunity were the number of T cells [1–9], the ratios of leukocytes subsets [3–8] and the proliferation response to lectins [1,2,5,6,8]. Since none of these indicators is antigen-specific, they do not always reflect antigen-specific immunity. Therefore, it remains unclear how antigen-specific immunity is affected by CPB and steroid administration during surgery. We thus investigated the effect on the T cell response to purified protein derivative (PPD) antigen by CPB and steroid administration. In addition, we first assessed the antigen presentation ability after open-heart surgery by using a cross-stimulation system.

2. Material and methods

Twenty adult patients who underwent elective cardiac surgery with cardiopulmonary bypass (CPB) consecutively at Kyushu University Hospital were studied. These patients were randomly divided into two groups; for group A (10 patients), steroid (hydrocortisone 50 mg/kg) was administered immediately before and after surgery with CPB, regardless of steroid administration. For group B (10 patients), steroid administration was not administered. To assess the effect of surgical injury of antigen-specific immunity, eight patients who underwent off-pump CABG were studied as group C. All patients gave their written informed consent. This study was approved by our institutional ethics committee. They did not have any major complication such as kidney, suprarenal gland
function, or liver dysfunction. The patient profiles for the three groups are shown in Table 1.

2.1. Intraoperative patient management

Standard anesthetic (fentanyl; 50 μg/kg, midazoram; 1–2 mg/body pancuronium; 0.1 mg/kg) and monitoring techniques (electro-cardiogram, central/pulmonary and arterial pressure monitoring, urine output, bladder and skin temperature monitoring) were used in all patients. The circuit was primed with 1600 ml of Ringer’s lactate solution, 100 ml of 25% human albumin, 45 mEq of sodium bicarbonate, 1000 mg of vitamin C, 150 ml of 20% of mannitol, 4500 U of heparin and 1 g of cefazolin. Before the institution of CPB, heparin was administered (300 IU/kg), and activated coagulation time was kept at more than 400 s. A CPB flow rate of 2.5 l/min per m² was maintained. During the CPB procedure, the lowest bladder temperature was kept around 30 °C. The blood pressure was kept over 50 mmHg using phenylephrine. Protamine was given (300 IU/kg) at the end of CPB.

2.2. Blood sampling

Blood samples were taken from patients just before the induction of anesthesia (Pre), at the end of surgery (Post), and on days 1, 3 and 7 post-operation (POD1, POD3 and POD7). The peripheral blood mononuclear cells (PBMCs) were separated from the heparinized blood sample by Ficoll-Hypaque (Pharmacia Biotech) gradient centrifugation (for 30 min at 2000 rpm.). The PBMCs were suspended in RPMI1640 medium (GIBCO, BRL) containing 10% human serum and 10% DMSO and then stocked at −80 °C until functional assay could be carried out.

2.3. Analysis of T cell response to PPD antigen

After all samples were collected, T cell responses to PPD antigen were examined by 3H-thymidine uptake; 1 £ 10⁵ PBMCs suspended in 100 ml RPMI1640 containing 10% human serum was put in each well to 96-well U-bottom microplate. PPD antigen was then added to each well to a final concentration of 5 μg/ml. Quadruplicate assays were then performed. The cells were incubated at 37 °C for 6 days and then 20 μCi of ³H-thymidine was added and followed by another 12-h incubation at 37 °C. The radioactivity precipitated on filter paper was measured in a liquid scintillation counter.

2.4. Cross-stimulation system

PBMCs from Pre or POD 1 were incubated in RPMI1640 containing 10% human serum with PPD antigen at 5 μg/ml at 37 °C for 3 h. These antigen-loaded PBMCs were irradiated at 3000 rad and vigorously washed three times; 1 £ 10⁵ of these cells were put in each well as APCs and then naive PBMCs from Pre or POD 1 were added as responder cells. The cells were incubated at 37 °C for 6 days and then 20 μCi of ³H-thymidine was added and followed by another 12-h incubation at 37 °C. The radioactivity precipitated on filter paper was measured in a liquid scintillation counter.

2.5. Data analysis

The Fisher exact tests were applied to categorical data. The kinetic data in the three groups were first analyzed by the two-way repeated measure analysis of variance (ANOVA). A Scheffe-type multiple comparison test was used to compare the perioperative T cell responses. A probability value of less than 0.05 was considered significant.
3. Results

3.1. Impairment of antigen-specific immunity by surgical injury, cardiopulmonary bypass and steroid administration

There were no deaths or significant complications such as mediastinitis or pneumonia in the three groups. There was no significant difference in the numbers of PBMCs among the three groups during the perioperative period (Fig. 1). The T cell responses to PPD was significantly impaired after surgery and significant difference was found between groups [ANOVA: $P$ (group) = 0.044, $P$ (time) $<$ 0.001, $P$ (interaction) = 0.027 for the cpm values and $P$ (group) $<$ 0.001, $P$ (time) $<$ 0.001, $P$ (interaction) = 0.0035 for the percent changes] (Fig. 2). Antigen-specific T cell responses decreased to about 5% of the preoperative values immediately after surgery with CPB, regardless of steroid administration (Fig. 2). The T cell response in group B on POD 7 was significantly higher than that in group A (Fig. 2). This indicated that steroid administration retarded the recovery of antigen-specific immunity.

3.2. Effect on T cell function and antigen-presentation by CPB and steroid administration

Regarding the cross-stimulation system, the proliferative ability of the T cells and the antigen presentation ability of the APCs decreased to 30% and 31% in group A, and 45% and 80% in group B on POD 1 (Fig. 3A,B). These results indicated that steroid administration markedly enhanced the impairment of both the antigen presentation ability of APCs and the proliferative ability of T cells. In group C, the proliferative ability of the T cells and the antigen presentation ability of APCs decreased to 60% and 85% of preoperative levels, respectively (Fig. 3C). Comparing the results of group B and group C in Fig. 3, the proliferation ability of T cells was more sensitive to the damage by CPB than antigen presentation ability.

4. Discussion

Several investigators have reported that cell-mediated immunity is impaired by cardiac operations using cardiopulmonary bypass [1–9]. However, these studies used the proliferation response of T cells to lectins such as Phytohemagglutinin (PHA) as a marker of cell-mediated immunity. The proliferation response of T cells to a lectin is not antigen-specific, and only reflects the proliferative ability of both naive and memory T cells. Therefore, using this as an indicator of antigen-specific immunity against a pathogen, the immunity against the pathogen may be overestimated, due to compensation by newly-entering naive T cells that do not possess immunity against the pathogen. It thus remains unclear how open-heart surgery affects the antigen-specific immunity. We showed in this paper that antigen-specific immunity after open-heart surgery was impaired much more than we estimated by antigen-non-specific T cell responses (T cell responses to PHA was about 25% of the preoperative values on POD 1 [8], while T cell responses to PPD antigen was about 5%). Moreover, we cannot assess the effect of open-heart surgery on the antigen-presentation ability of APCs based on previous reports, because the proliferation response of T cells to lectin does not require an antigen-presentation process. This study, therefore, is the first to evaluate the effect of CPB on antigen presentation. We showed that CPB impaired the T cell function much more than the APC function and that steroid enhanced the impairment of the both functions.

Steroid administration has been reported to reduce the inflammatory response during CPB [10,11] and to improve the postoperative course [12,13]. Some reports, however, failed to show any favorable effects of steroid in open-heart surgery [14,15]. We previously reported that steroid administration synergistically suppressed the T cell response to PHA [8]. To reduce the adverse effect of steroid administration, we changed intermediate-acting methylprednisolone (biological half life: 12–36 h) to short-acting hydrocortisone (biological half life: 8–12 h). Because anti-inflammatory power of methylprednisolone is fivefold stronger than that of hydrocortisone and we increased the dose of 20 mg/kg to 50 mg/kg of hydrocortisone, which is equivalent to 10 mg/kg of methylprednisolone as for anti-inflammatory effect. However, we have shown in this report that even short-acting steroid has long suppressive effect on antigen-specific immunity.
We did not show the population change of PBMCs. Nguyen et al. showed that the number of monocytes did not change significantly during the perioperative period [4]. Thus the decrease of antigen-presenting ability, as shown in Fig. 3, is interpreted to indicate impaired monocyte function.

Most Japanese have immunity against PPD antigen, which is derived from *Mycobacterium tuberculosis* through regular vaccination. We therefore chose this antigen so that we did not need to immunize patients before their operation. The current study design is thus only possible in countries that perform vaccination for tuberculosis, such as in Japan.

The limitation of this study was the small number of patients and the heterogeneity of the operative procedures. However, we could not find any differences among patients who underwent different surgeries, indicating that the operative procedure itself was not a major factor in the impairment of adaptive immunity. Therefore, if all patients had undergone CABG, the results would not change significantly.

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**Fig. 2.** (A) T cell responses to purified protein derivative (PPD) antigen during the perioperative period. Open diamonds indicate group A, closed squares and open triangles indicate group B and group C, respectively. The data were shown as counts per minute (cpm). (B) Percent changes of T cell proliferative responses to PPD antigen during the perioperative period. Symbols indicate the same groups as in (A). The data were expressed as percentages compared to preoperative value.

**Fig. 3.** Assessment of the effect on antigen-presentation and T cell function by cardiopulmonary bypass and steroid administration. Irradiated PBMCs loaded with PPD antigen obtained from Pre or POD1 were prepared as antigen-presenting cells (APCs), and then were added to naive PBMCs obtained from Pre or POD1 as responder T cells. Data are expressed as mean percentages of the response of preoperative T cells stimulated by preoperative APCs (the first column).
5. Conclusions

Open-heart surgery with CPB severely impaired antigen-specific immunity, regardless of steroid administration. Steroid administration during surgery retarded the recovery of antigen-specific immunity. This may indicate that steroid administration in open-heart surgery increases the incidence of infection in the late phase of the postoperative period.

References