

## Cytokine and Prostaglandin E<sub>2</sub> Release from Leukocytes in Response to Metal Ions Derived from Different Prosthetic Materials: An In Vitro Study

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**Abstract:** Cytokines produced by leukocytes in the periprosthetic membranes surrounding joint replacements have been implicated as causal agents in osteolysis and prosthetic loosening. In this study, we used an in vitro leukocyte culture system to monitor the response of leukocytes to various metal ions and their possible roles in the mechanism of aseptic loosening. Human peripheral leukocytes were isolated and incubated with various concentrations of Co<sup>2+</sup>, Cr<sup>3+</sup>, and Ti<sup>3+</sup> ions. Leukocyte cell counts and the levels of the tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), interleukin-6 (IL-6) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) released into the media were analyzed at 1 h, 3 h, and 1, 3, and 7 day intervals. The results showed that adding different metal ions into leukocyte cultures did not affect the cell counts. Exposure of leukocytes to Co<sup>2+</sup>

ion increased the release of TNF- $\alpha$ , IL-6, and PGE<sub>2</sub>. Exposure of leukocytes to Cr<sup>3+</sup> ion did not increase the release of TNF- $\alpha$  but increased the secretion of IL-6 and PGE<sub>2</sub>. In contrast, exposure of the leukocytes to Ti<sup>3+</sup> ions was associated with a decrease in the release of TNF- $\alpha$  and PGE<sub>2</sub> and a minimal change in IL-6 noted after 7 days' culture. The present study elucidated the possible mechanisms involved in periprosthetic osteolysis and the inflammatory response of human leukocytes to metal ions. We found that cobalt ion is the most potent stimulant for cytokines and prostaglandin secretion by leukocytes. This elucidation, in combination with other efforts to reduce the generation of wear debris and metal ions, may improve the longevity of orthopedic implants. **Key Words:** Leukocytes—Metal ions—Cytokines—Prostaglandins.

Metals and metal alloys have a wide range of applications as prosthetic materials for bone tissue reconstruction. The most common metal alloys used in orthopedics are titanium alloy and cobalt-chromium alloy. Corrosion and fretting have been reported to occur at the head-neck interface in the modular hip implant (1). Substantial evidence suggests that the adverse tissue response to prosthesis wear particles is an important contributor to bone loss around implants and loosening of the prosthesis (3). Cement, polyethylene, the high surface area associated with porous-coated implants (2), and metallic particulate

wear debris may contribute to the pathogenesis and progression of osteolysis (3). The appearance and tissue response around any given prosthesis is related to the balance among the rate of production of wear particles, the ability of the tissues to deal with the particles, and the rate of clearance of the particles from the joint.

The high surface area associated with the porous-coated implant (2) and metallic wear debris (3) increases the potential for ion release into the physiological environment. In addition to abundant ultrahigh molecular weight polyethylene wear debris and bone cement, disruption of the passive oxide layer during dynamic loading conditions also contributes to ion release (4). A relationship between immune responses to metallic particulate wear debris and loosening of orthopedic implants has been explored (5,6). Several groups have measured the

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amount of metal released from orthopedic alloy into blood and urine (7,8), organs (8,9), and the local tissue environment (10). The minimal changes in the concentration of metal ion in serum, urine, spleen, and lung tissue indicate that species released from metal implants by passive dissolution have low solubility. Local tissue levels of ions are usually significantly elevated (11). Even with the use of corrosion resistant alloy such as Ti6Al-4V and Co-Cr-Mo, ion release from orthopedic implants remains a concern.

At the interface of the loose prosthesis, macrophage phagocytosing particles are usually seen in close proximity to osteoblasts, which are known to be a response to mediators released from macrophages and also to be important in bone resorption (12). Previous studies have examined the effects of particulates and metal ions on various types of cells, such as osteoblasts, fibroblasts, and synovial cells. Relatively little research has been done concerning the effects of metal ions upon leukocytes. If ions exert toxic effects upon bone-forming cells in the periprosthetic environment, biological responses of leukocytes to metals' ion may play a role in loosening of orthopedic implants. The purpose of this study was to investigate the acute responses of metal ions derived from commonly used orthopedic implants on leukocytes.

## MATERIALS AND METHODS

### Cell culture

Human leukocytes were isolated from the peripheral blood of healthy individuals. All individuals were screened by questionnaire to eliminate inflammatory conditions and by serum testing to eliminate blood borne pathogens. Forty-five ml of heparinized blood samples was carefully added to the top of 5 ml of Hesperan (6% hetastarch in 0.9% NaCl, 310 mosm., NPBI, Emmer-Compascuum, The Netherlands for DuPont Pharmaceuticals, Wilmington, DE, U.S.A.) and left for 15 min until the leukocytes had separated. Lysis of red cells was performed by the hypotonic method. Briefly, the upper leukocyte concentrate was added to distilled water for 10 s; then, the same volume of 2× phosphate-buffered saline (PBS) solution was added immediately. The leukocyte concentrate was centrifuged and further washed with PBS solution, mixed with Dulbeccos' modified Eagles' medium (DMEM) and counted by Microcellular Counter F-500 (Sysmex Corp., Kobe, Japan). In the experiments, 3 ml of leukocyte cultures was seeded into six 30 × 30 mm tissue culture wells (seedling density of  $1 \times 10^5$  cells/well). The culture media used was DMEM supplemented with ITS (insulin, 10

μg/ml; transferrin 10 μg/ml, sodium selenite  $5 \times 10^{-3}$  μg/ml; Sigma Corp., St. Louis, MO, U.S.A.), penicillin G sodium, 100 U/ml and streptomycin 100 μg/ml (Gibco BRL, Grand Island, NY, U.S.A.). The media were mixed with various types of metal ions as described below. The dishes were incubated at 37°C in an atmosphere supplemented with 5% CO<sub>2</sub>. The day of plating was considered as the zero day of culture. The test media were removed from wells at 1 h, 3 h, and 1, 3, and 7 days, and stored frozen at -80°C in 500 μl aliquotes until further analysis. The levels of the cytokines released into the media were measured using commercially available immunoassay kits as described below.

### Ion solutions tested

Three major metal ions, Co<sup>2+</sup>, Cr<sup>3+</sup>, and Ti<sup>3+</sup> were selected for this study. Single ion solutions were prepared from atomic absorption standards (Sigma Corp., St. Louis, MO, U.S.A.). The standards consist of metal in various forms: pure, oxides, dichromates, and nitrates. Control experiments demonstrated that, at equimolar concentrations, nitrate counter ions did not affect cellular responses. When needed, the pH of the medium was neutralized following addition of ions. Stock solutions were prepared in sterile water and then diluted such that 10 μl would provide the desired final concentration in 200 μl of DMEM culture medium. Pilot experiments showed that addition of these concentrations altered pH by < 0.05, and induced no precipitation. Concentration spanning three orders of magnitude, from 0.1 ppm, 1.0 ppm, and 10 ppm, were used. The concentrations in molar quantities of 1 ppm of Ti<sup>3+</sup>, Co<sup>2+</sup>, and Cr<sup>3+</sup> were  $4.89 \times 10^{-9}$ ,  $1.70 \times 10^{-8}$ , and  $1.92 \times 10^{-8}$  M, respectively. Concentrations are predominantly expressed in parts per million, rather than micromolarity, to facilitate comparison with data for ion concentrations in body fluids and tissues.

At 1, 3 and 6 h, and at 1, 3, and 7 days of culture, leukocytes were visually examined by inverted microscopy (Nikon, Tokyo, Japan), and assays performed for the cytokines and for PGE<sub>2</sub>. Cellular responses of treated samples were compared with control samples, that is, those not exposed to ions, and expressed as percentages of the control values.

### Total cell number

At the end of experiments, the attached cells were passaged by trypsin-ethylenediaminetetraacetate (EDTA), washed with PBS solution, mixed with DMEM, and counted by Microcellular Counter F-500 (Sysmex Corp., Kobe, Honshu, Japan). Absolute numbers of the leukocytes were accurately measured.

### Analyses of TNF- $\alpha$ , IL-1, IL-6, and PGE<sub>2</sub> in culture media

The production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), and interleukin-6 (IL-6) in culture medium were analyzed by enzyme-linked immunosorbent assay (ELISA) methods with commercially available assay kits (R & D System Inc., Minneapolis, MN, U.S.A.). The production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in culture medium was also analyzed by the ELISA method using the kit supplied by Assay Designs, Inc. (Ann Arbor, MI, U.S.A.). A Microelisa reader (Emax Science, Sunnyvale, CA, U.S.A.) was used to quantitate product formation in the ELISAs.

### Statistical analysis

The differences of cytokines or PGE<sub>2</sub> in media incubated with the various tested metal ions compared with control samples were evaluated by an unpaired Students *t*-test statistical method. A significant level was applied when *p* value < 0.05 was observed.

## RESULTS

### Cell count

Even at the concentration of 10 ppm, the addition of metal ions to the culture medium did not show any evidence of decreases in leukocyte cell numbers. Cell populations of control and various metal ions preparations at 1 or 3 h, and 1, 3, or 7 days were all not statistically significant (*p* > 0.05).

### Effect of metal ions on cytokine secretion

The TNF- $\alpha$  secretion by leukocytes was highest at all time points tested when cultured with medium containing the 3 different Co<sup>2+</sup> ion concentrations. When compared with the control sample, there was an increase in TNF- $\alpha$  concentration when leukocytes were cultured with 0.1 ppm Co<sup>2+</sup> for 1 h (*p* = 0.05). Significant increases in TNF- $\alpha$  concentration were also observed with 0.1 ppm Co<sup>2+</sup> ion in the medium and leukocytes cultured for 3 h (*p* = 0.006), 1 day (*p* = 0.01), 3 days (*p* = 0.001), and 7 days (*p* = 0.034). Similar results were found when leukocytes were cultured with 1.0 ppm, and 10 ppm Co<sup>2+</sup> ion (Fig. 1). If the leukocytes were cultured with the Ti<sup>3+</sup> ion, there were also significant differences in the TNF- $\alpha$  secretion. However, the TNF- $\alpha$  secretion was significantly lower than that of controls (Fig. 1). There was no statistically significant difference in TNF- $\alpha$  secretion when leukocytes were cultured with medium containing the 3 concentrations of Cr<sup>3+</sup> ion (Fig. 1).

The secretion of IL-6 by leukocytes was not so obvious as that of TNF- $\alpha$ . When cultured with Co<sup>2+</sup>,

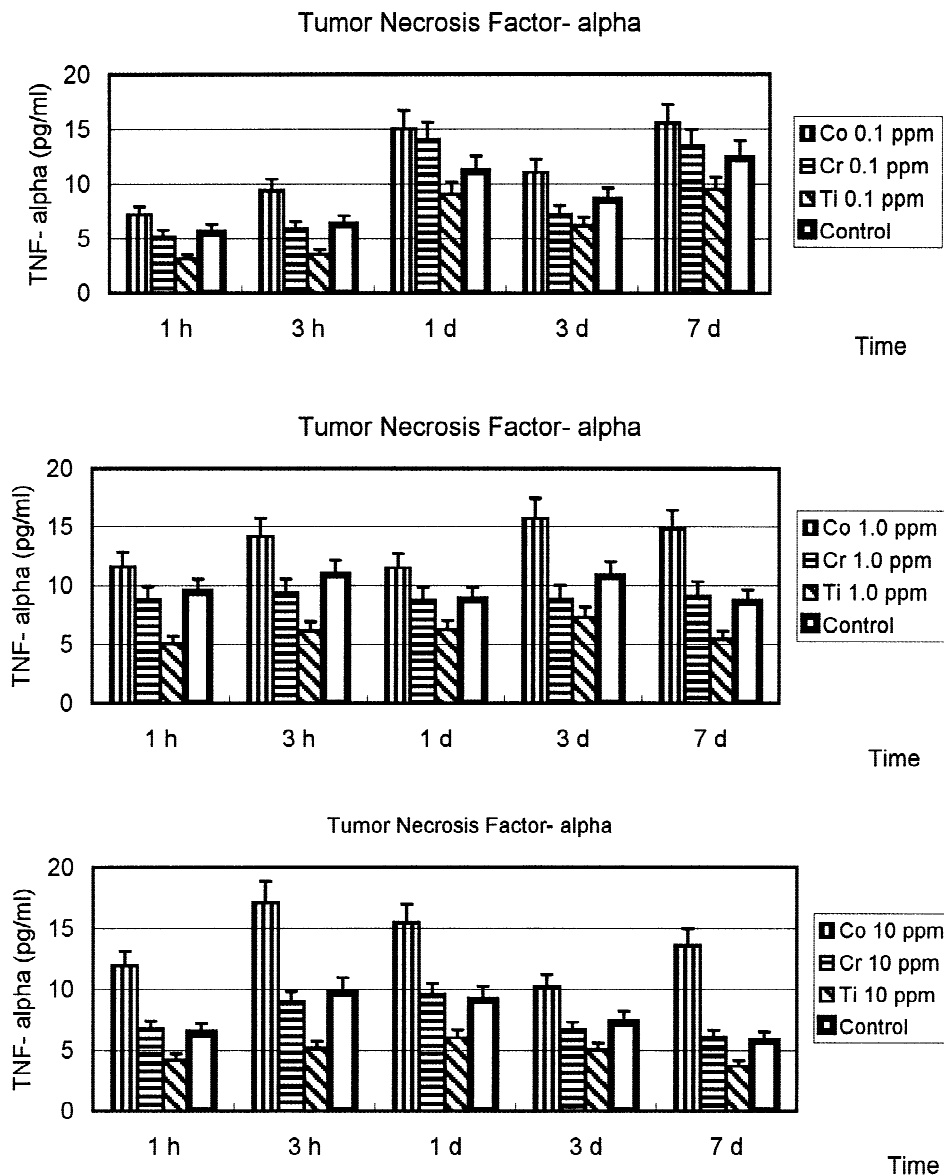
Cr<sup>3+</sup>, or Ti<sup>3+</sup> ion at the concentration of 0.1 ppm, the IL-6 secretion by the leukocytes was not significantly different to that of control samples. When leukocytes cultured with 1.0 ppm Co<sup>2+</sup> for 7 days, there was a significant increase in IL-6 secretion (*p* < 0.001) (Fig. 2). As the concentration of Co<sup>2+</sup> increased to 10 ppm, the change in IL-6 concentration appeared earlier at 3 h (*p* = 0.005) and 1 day (*p* = 0.026), but disappeared after 3 days' culture (Fig. 2). When leukocytes were cultured with Cr<sup>3+</sup> or Ti<sup>3+</sup> ion at concentrations of 1.0 ppm or 10 ppm for 7 days, there were also significant changes in the IL-6 secreted by leukocytes. The IL-6 secretion was significantly lower than that of the control (Fig. 2). When leukocytes were cultured in media with metal ions, there is no significant effect on IL-1 secretion by any ion at any concentration.

### Effect of metal ions on prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) secretion

The secretion of PGE<sub>2</sub> by leukocytes was also most obvious when leukocytes were cultured with Co<sup>2+</sup> ion. At the 0.1 ppm concentration, the secretion of PGE<sub>2</sub> by leukocytes was not different to that of the control samples. The difference in PGE<sub>2</sub> secretion was significantly increased when leukocytes were cultured with 1.0 ppm Co<sup>2+</sup> ion for 7 days (*p* = 0.016). When leukocytes were cultured with 10 ppm Co<sup>2+</sup> ion, PGE<sub>2</sub> was significantly increased compared with the control samples as early as after 1 h culture (*p* < 0.001) and persisted to the end of 7 days' culture (Fig. 3). When leukocytes were cultured with Cr<sup>3+</sup> ion at the concentration of 10 ppm, the difference in PGE<sub>2</sub> secretion reached a significantly higher level compared with controls after 1 day's culture (*p* = 0.02) and persisted to the end of 7 days' culture. Leukocytes cultured with Cr<sup>3+</sup> ion at the concentration of 10 ppm significantly decreased secretion of PGE<sub>2</sub> before 3 h of culture (Fig. 3).

### Time effect on different concentrations of metal ions

Different concentrations of metal ions exerted their maximal effects on leukocytes at different time intervals. For the Co<sup>2+</sup> ion, the higher concentrations in the medium resulted in higher amounts of TNF- $\alpha$  earlier and which persisted longer; however, similar results were not observed for the Cr<sup>3+</sup> and Ti<sup>3+</sup> ions (Fig. 1). In contrast, IL-6 secretion for 10 ppm Co<sup>2+</sup> ion in culture media reached a peak at 3 h and then decreased gradually. For 1.0 ppm Co<sup>2+</sup> ion in culture media, the IL-6 secretion reached a peak at 7 days and for 0.1 ppm Co<sup>2+</sup> ion in culture media, there was no statistically significant difference (Fig. 2). PGE<sub>2</sub>



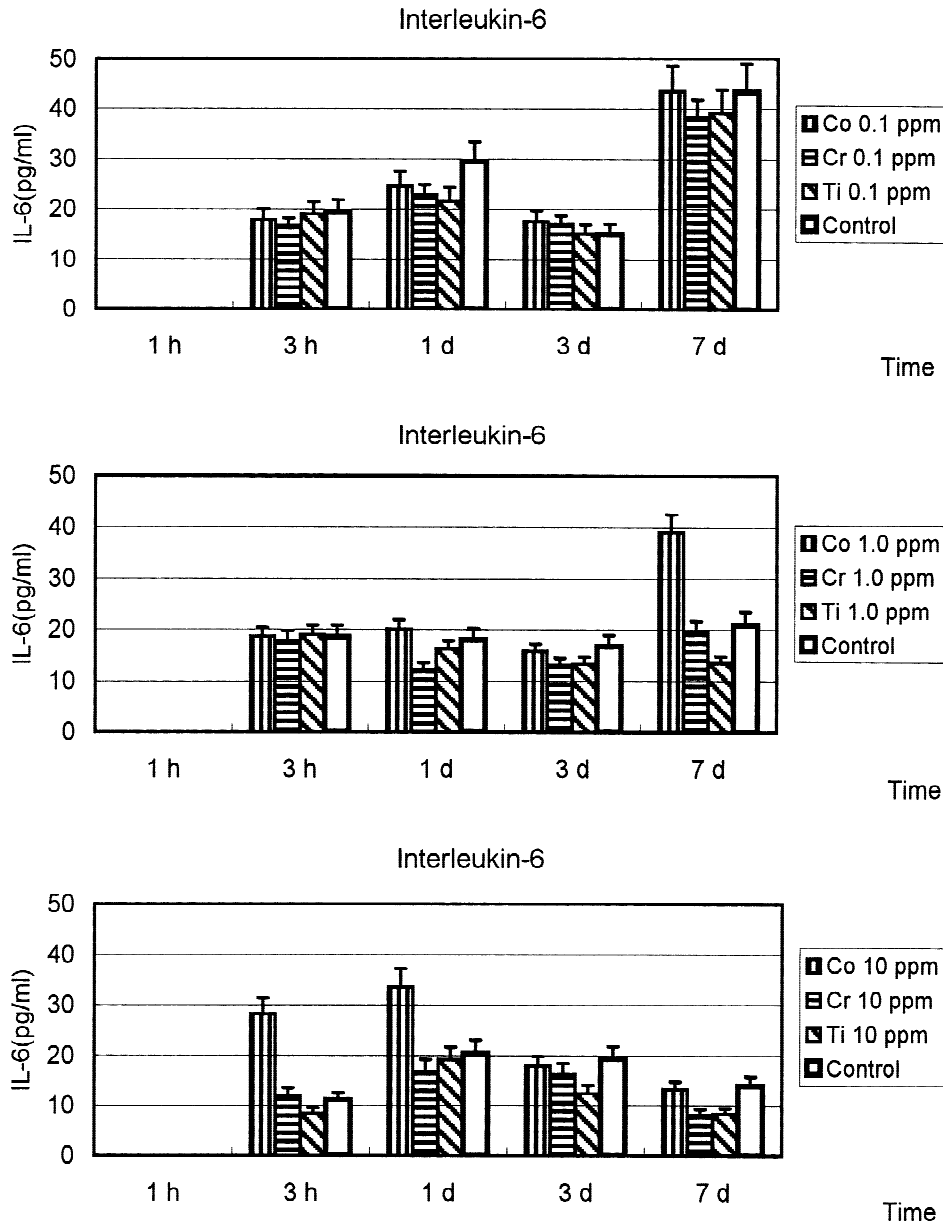
**FIG. 1.** Shown are changes in secretion of TNF- $\alpha$  by leukocytes when cultured with different metal ions for periods up to 7 days. For levels of the analytes tested the mean quantities  $\pm$  SE are plotted. TNF- $\alpha$  quantities in culture media were consistently higher for leukocytes cultured in media containing the 3 concentrations of Co<sup>2+</sup> ion, and consistently lowest for leukocytes in media containing the 3 Ti<sup>3+</sup> ion concentrations. When compared with the control values, significantly higher quantities of TNF- $\alpha$  were observed for the 0.1 ppm Co<sup>2+</sup> ion-medium at 1 h ( $p = 0.05$ ), 3 h ( $p = 0.006$ ), 1 day ( $p = 0.01$ ), 3 days ( $p = 0.001$ ), and 7 days ( $p = 0.034$ ). Similar results were observed for TNF- $\alpha$  quantities of leukocytes cultured in media containing the 1.0 ppm and 10 ppm Co<sup>2+</sup> ion concentrations. For leukocytes cultured on media containing Ti<sup>3+</sup> ion, TNF- $\alpha$  quantities were significantly less compared with the control cultures for all time points and Ti<sup>3+</sup> ion concentrations. Leukocytes cultured in medium containing the 3 different concentrations of Cr<sup>3+</sup> ion exhibited no significantly different quantities of TNF- $\alpha$  at all time points compared with the control leukocyte cultures.

secretion was also highest in cultures with Co<sup>2+</sup> ion. With 1.0 ppm Co<sup>2+</sup> ion in the media, PGE<sub>2</sub> secretion increased to a statistically significant level at 7 days' culture. With 10 ppm Co<sup>2+</sup> or Cr<sup>3+</sup> ion in the media, the changes in PGE<sub>2</sub> secretions reached their highest levels at 3 days' culture (Fig. 3).

## DISCUSSION

Corrosion resistance of titanium alloys and Co-Cr alloy is considered to be related to the ability of these materials to form an oxide layer that may be involved in supporting the initial attachment and growth of osteoblast-like cells (13-16). A number of investigations have demonstrated that metal ions can be released from metallic implants as the result of

corrosion (17). It seems that the cytotoxicity induced by metal ions is affected by their concentration (18), the time of exposure to these ions, and what kind of cells are exposed (19-21). The biological response of released metal ions and alloys on the bone tissues have been extensively studied in vitro and in vivo (22-24). Although these studies provide valuable information about cellular responses to implant materials, there are many experimental problems which need to be solved. In order to identify a material as biocompatible to bone, it is more informative to pay attention to the effect of the material on specific cellular functions than to evaluate its general cytotoxicity. When using the alloy, it is difficult to determine which element of the alloy is responsible for the adverse action. The conventional cytotoxicity



**FIG. 2.** Changes in secretion of interleukin-6 (IL-6) by leukocytes when cultured with different metal ions for times up to 7 days were recorded. For levels of the analytes tested the mean quantities  $\pm$  SE are plotted. IL-6 quantities in culture media were higher for leukocytes cultured in media containing  $\text{Co}^{2+}$  ion at the 1.0 or 10.0 ppm concentrations and lower for leukocytes in media containing the  $\text{Ti}^{3+}$  or  $\text{Cr}^{3+}$  ion at the 1.0 or 10.0 ppm concentrations. When compared with the control values, significantly higher quantities of IL-6 were observed for the 1.0 ppm  $\text{Co}^{2+}$  ion-medium at 7 days ( $p < 0.001$ ) and 10 ppm  $\text{Co}^{2+}$  ion-medium at 3 h ( $p = 0.005$ ) and 1 day ( $p = 0.026$ ). For leukocytes cultured in media containing 10 ppm  $\text{Co}^{2+}$  ion, the changes in IL-6 quantities were not seen at 3 days' culture. IL-6 quantities in culture media were lower for leukocytes cultured in media containing the 1.0 or 10 ppm concentrations of  $\text{Ti}^{3+}$  or  $\text{Cr}^{3+}$  ion for 7 days ( $p < 0.05$ ) when compared with control samples. For leukocytes cultured on media containing  $\text{Ti}^{3+}$  or  $\text{Cr}^{3+}$  ions at 0.1 ppm concentrations, IL-6 quantities were not significantly different to that of the control cultures for all time points.

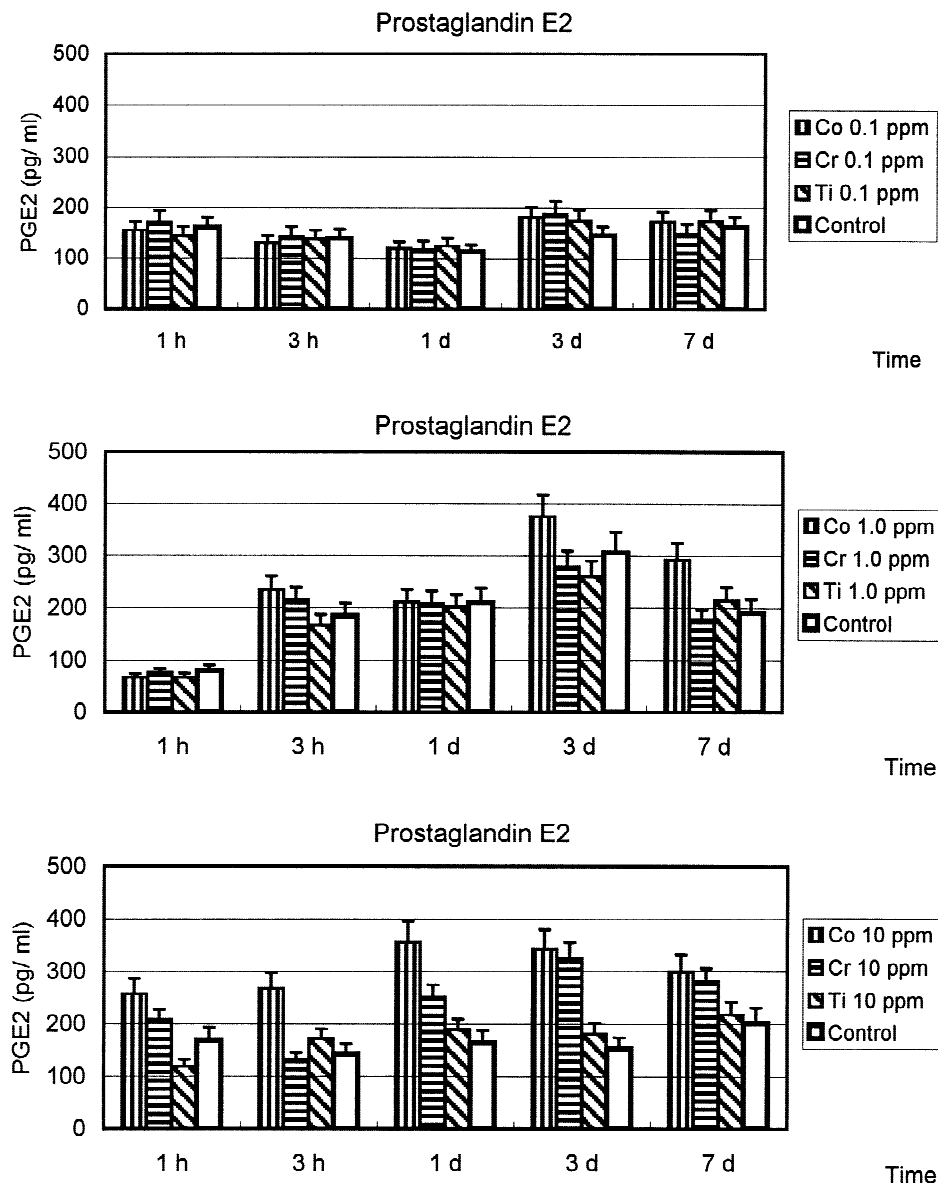
evaluation usually focuses on the viability of cells, but does not explain the material's influence on specific cellular functions and differentiation processes. In this study, we evaluated the effect of  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ , and  $\text{Ti}^{3+}$  ions, which may be released from metallic implants in vivo (17,25), on leukocyte metabolism.

In the preliminary study of our institute, we measured the ion concentration in the serum of patients with aseptic loosening. The serum concentration of  $\text{Ti}^{3+}$  ion was 319.6 ppb (0.32 ppm) in the patients with aseptic loosening whereas only 5.8 ppb in patients without evidence of aseptic loosening. The serum concentrations for  $\text{Cr}^{3+}$  and  $\text{Co}^{2+}$  were 108.1 ppb and 116.1 ppb for the aseptic loosening whereas

the control sera were 5.8 ppb and 0.9 ppb, respectively (26). In the patients with aseptic loosening, their peripheral leukocytes must be chronically exposed to low levels of ions released from the implants, and the local metal ion concentration must be much higher than that of the serum level. In this study, we elucidated the effects of 0.1 ppm, 1.0 ppm and 10 ppm of  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ , and  $\text{Ti}^{3+}$  ions on the leukocytes.

The cytotoxicity induced by metal ions is affected by their concentration, by the time of exposure to these ions, and what kind of cells are exposed. It is reported that the LD-50% for bone marrow stroma cells of rats were 0.4 ppm for  $\text{Cr}^{3+}$ , 2 ppm for  $\text{Co}^{2+}$ ,





**FIG. 3.** Changes in secretion of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by leukocytes when cultured with different metal ions for periods up to 7 days are presented. For levels of the analytes tested the mean quantities ± SE are plotted. PGE<sub>2</sub> quantities in culture media were higher than control samples for leukocytes cultured in media containing 1.0 or 10 ppm of Co<sup>2+</sup> ion and 10 ppm Cr<sup>3+</sup> ion. When compared with the control values, significantly higher quantities of PGE<sub>2</sub> were observed for the 1.0 ppm Co<sup>2+</sup> ion-medium at 7 days (*p* = 0.016). Similar higher quantities of PGE<sub>2</sub> were observed for leukocytes cultured in media containing the 10 ppm Co<sup>2+</sup> ion concentrations at all time points tested (*p* < 0.001). When leukocytes were cultured with Cr<sup>3+</sup> ion at the concentration of 10 ppm, the quantities of PGE<sub>2</sub> were significantly lower at 3 h of culture; then, the quantities of PGE<sub>2</sub> reached a significantly higher level after 1 day's culture (*p* = 0.02) and persisted to the end of 7 days' culture.

and 1.5 ppm for Co–Cr–Mo alloy (18). Different kinds of cells have different response to metal ions. At a concentration of 10 ppm for Co<sup>2+</sup> ion, 50 ppm for Ni<sup>2+</sup> ion, or 10 ppm for Cr<sup>3+</sup> ion, the metal ions did not cause any lethal effects on human leukocytes (19,20). At a concentration of 5 ppm, Co<sup>2+</sup> ions will induce lethal effects on fibroblasts (27), and the Ni<sup>3+</sup> ion can inhibit the growth of fibroblasts at a concentration of 7.5 ppm (21). In this study, even 10 ppm of Co<sup>2+</sup>, Cr<sup>3+</sup>, or Ti<sup>3+</sup> ions did not cause significant cytotoxicity on human leukocytes and there were no significant changes in the cell numbers of various tests.

In 1997, Sun et al. demonstrated that the cytotoxicity rank order of several metal ions by ROS 17/2.8 osteoblast-like cell was V<sup>3+</sup> > Ti<sup>4+</sup>, Co<sup>2+</sup> > Ni<sup>2+</sup> >

Cr<sup>3+</sup>, Al<sup>3+</sup> (28). Wataha demonstrated that the cytotoxicity rank order of several metal ions by Balb/C3T3 cell line was V<sup>3+</sup> Co<sup>2+</sup> > Ni<sup>2+</sup> > Ti<sup>4+</sup> > Cr<sup>3+</sup>, Al<sup>3+</sup> (22). In this study, this rank is not the same. For the concentration of 0.1 ppm Co<sup>2+</sup> ions, the TNF-α secretion attained a significant level at 3 h culture; for 1.0 and 10 ppm, the TNF-α secretion attained a significant level even at 1 h culture (Fig. 1). For the Co<sup>2+</sup> ion, the changes in TNF-α titer did not attain a significant level at any concentration whereas for the Ti<sup>3+</sup> ion, the TNF-α secretion was even lower than that of the control samples (Fig. 1). The order for TNF-α secretion by leukocytes is Co<sup>2+</sup> > Cr<sup>3+</sup> or Ti<sup>3+</sup>. This order suggests that human leukocytes are more sensitive to the Co<sup>2+</sup> ion than the Cr<sup>3+</sup> ion or Ti<sup>3+</sup> ion.

None of 3 three ions stimulated leukocytes to secrete IL-1 to a statistically significant level, but there was a significant change in the IL-6 level. The first significant effect was observed for  $Co^{2+}$  at the concentration of 1.0 ppm and 7 days' culture. At the concentration of 10 ppm  $Co^{2+}$ , the IL-6 secretion attained a significantly higher level at 3 h culture, reached its peak at 1 day, then decreased to a non-significant level thereafter compared with controls (Fig. 2). The reason for variation in the IL-6 secretion is not known, but the exposure time may play a key role in the response of leukocytes to metal ions. IL-6 is secreted by monocytes/macrophages, and in vivo, the physiologic response of macrophages is slower than that of leukocytes. This fact can partially explain why the secretion of IL-6 is slower than that of TNF- $\alpha$ .

In 1993, Haynes et al. reported that titanium particles induced more release of  $PGE_2$  whereas cobalt-chromium particles induced more secretion of IL-6 (23). Different results were obtained in this study. The  $Co^{2+}$  and  $Cr^{3+}$  ions, induced significantly more release of  $PGE_2$  than did the  $Ti^{3+}$  ions, and this was true for a wide range of concentrations (Fig. 3). Exposure of the leukocytes to the  $Co^{2+}$  ion increased the release of TNF- $\alpha$ , IL-6, and  $PGE_2$ . Exposure of the leukocytes to the  $Cr^{3+}$  ion did not increase the release of TNF- $\alpha$  but increased the secretion of IL-6 and  $PGE_2$ . In contrast, exposure of the leukocytes to  $Ti^{3+}$  ions was associated with a decrease in the release of TNF- $\alpha$  and  $PGE_2$  with minimal change in IL-6 noted after 7 days' culture (Figs. 1-3). It has been reported that  $PGE_2$  can inhibit the secretion of IL-1 (27). This can partially explain the reason why the IL-1 concentration is not significantly increased in this study.

Some of the differences in findings between the present study and others in the literature can be attributed to differences in cell types, ion species, and culture media. It is likely that different types of cells will have different sensitivities to ions, depending on the function and location of the cells. The form and valence of the metal can also influence cytotoxicity results. The composition and serum content of the culture medium used will affect availability and transport of ions into the cells. Higher serum concentration can provide more transferrin and other molecules that are able to carry ions to cells. For specific ion-binding molecules, the degree of saturation of binding sites of these carriers will also affect the availability of metal ions (29).

The metal ion concentrations around orthopedic implants are in the range that may induce an inflammatory reaction. To determine whether transient or

short-term exposure to ions was sufficient to induce inflammatory reaction, leukocytes were exposed to ions for increasing duration. For the  $Co^{2+}$  ions examined, TNF- $\alpha$  secretion was observed after just a 3 h exposure indicating that the  $Co^{2+}$  ions may rapidly become associated with the cells. The ability of  $Co^{2+}$  ion to induce TNF- $\alpha$  might be a major concern because this cytokine has the potential not only to reduce the numbers of bone forming cells but also to stimulate bone resorption (30).

Metal ions derived from corrosion of particulate wear debris from prosthetic materials can activate tissue macrophages to release a variety of inflammatory mediators. Among these cellular mediators, proinflammatory cytokines (IL-6 but not IL-1), TNF- $\alpha$ , and  $PGE_2$  are believed to be the most important components that provoke cell proliferation and stimulate osteoclasts to resorb the adjacent bone (31-35). The results of the present study demonstrate the acute reaction of leukocytes to metal ions. Certain metal species that are constituents of orthopedic implants can stimulate the release of a variety of inflammatory mediators even at concentrations down to the concentration of parts per billion which could partially explain the osteoporotic changes noted around a loosened prosthesis.

The corrosion at the metal surface of different prosthesis materials may produce different metal ions which will achieve higher concentrations around the implanted prosthesis. From this study, we found that metal ions derived from different prosthetic materials can stimulate human leukocytes to secrete osteoclastogenic cytokines and then possibly induce the aseptic loosening of the prosthesis. Cobalt ion is the most potent stimulant for cytokines and prostaglandin secretion by leukocytes. When leukocytes are cultured with metal ions, the IL-6 response is more obvious than that of IL-1; the possible mechanism is through the inhibitory effect of  $PGE_2$  on IL-1. Awareness of the importance of metal ions in cytokine-induced bone resorption will result in improvements in designs of implants and in operative techniques to reduce wear of components. The present study elucidated the biological mechanisms involved in periprosthetic osteolysis. The mechanism for the cause of aseptic loosening is multifactorial and is still not well known. Further study of the relationship between osteoclasts and metal ions is under investigation. These agents, in combination with other efforts to reduce the generation of wear debris, may improve the longevity of orthopedic implants.

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