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DR Haynes, SD Rogers, S Hay, MJ Pearcy and DW Howie
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The Differences in Toxicity and Release of Bone-Resorbing Mediators Induced by Titanium and Cobalt-Chromium-Alloy Wear Particles*

BY D. R. HAYNES, B.SC.†, S. D. ROGERS, B.SC.‡, S. HAY, B.APP.SCI.‡, M. J. PEARCY, B.SC., PH.D.‡,
AND D. W. HOWIE, M.B.B.S., PH.D., F.R.A.C.S.‡, ADELAIDE, SOUTH AUSTRALIA, AUSTRALIA

*Investigation performed at the Department of Orthopaedic Surgery and Trauma, Royal Adelaide Hospital,
and the Department of Pathology, University of Adelaide, Adelaide*

ABSTRACT: We investigated the relationship between the toxic effects of metal wear particles and their ability to stimulate the release of inflammatory mediators implicated in bone resorption. *In vitro* studies were carried out with use of rat peritoneal macrophages, which were exposed to either cobalt-chromium-alloy or titanium-aluminum-vanadium particles, milled from the metal components of hip prostheses. The particles were in the size-range of, and at concentrations similar to, those found in the tissues surrounding failed prostheses in humans. The titanium-aluminum-vanadium particles showed little toxicity even at high concentrations, while the cobalt-chromium particles were very toxic. The titanium-aluminum-vanadium particles induced significantly more release of prostaglandin E₂ than did the cobalt-chromium particles, and this was true for a wide range of concentrations. Exposure to titanium-aluminum-vanadium increased the release of prostaglandin E₂, interleukin-1, tumor necrosis factor, and interleukin-6. In contrast, exposure to cobalt-chromium particles was associated with a decreased release of prostaglandin E₂ and interleukin-6, and it had little effect on the release of interleukin-1 and tumor necrosis factor.

CLINICAL RELEVANCE: The results of this study demonstrate that there is a difference in the cellular

response to different types of metal-alloy wear particles that are of the same size. Cobalt-chromium particles are likely to be more toxic to periprosthetic tissue, but titanium-aluminum-vanadium particles are likely to cause release of more inflammatory mediators implicated in osteolysis. On the basis of these results, we believe that the release of titanium-aluminum-vanadium particles into the periprosthetic tissues may be worse than the release of equivalent numbers of cobalt-chromium particles. Therefore, special attention should be given to the design of titanium-aluminum-vanadium implants, particularly modular implants, so that the potential for wear is minimized.

The most common cause of failure of total hip and other arthroplasties is loosening of the prosthesis. There is now substantial evidence that an adverse tissue response to prosthetic wear particles is an important factor in the development of osteolysis and is a cause of loosening^{3,35,40}. Reports of black-staining around failed hip and knee prostheses of recent design have renewed interest in the role of metal-alloy wear particles in loosening and bone resorption around implants^{1,5}. The black-staining of tissues has commonly been shown to be due to large numbers of titanium-aluminum-vanadium-alloy wear particles³⁶, but cobalt-chromium-alloy wear particles are also present in large numbers around loose implants, as are stainless-steel particles³⁶. The presence of these wear particles and their possible role in bone resorption have been recognized since the development of metal-on-metal and metal-on-polyethylene implants for use in arthroplasties performed both with and without cement; however, since the early 1980's, much attention has been directed toward the adverse effects of polyethylene and polymethylmethacrylate particles^{13,18,19,27}. While both types of particles may be a major cause of osteolysis associated with wear particles, the contribution of metal-alloy wear particles, and the possible differences in the effect on cells by different alloys, deserve greater attention.

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†Department of Pathology, University of Adelaide, North Terrace, Adelaide, South Australia 5000, Australia.

‡Department of Orthopaedic Surgery and Trauma, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia.

Recent definitions of biocompatibility of implant materials have emphasized the importance of all potentially adverse effects of a material, not only toxicity³⁴. The importance of release of inflammatory mediators in response to particles has been appreciated. These mediators, particularly prostaglandin E₂, have been identified in human periprosthetic tissues^{11,25,31,38} and are released from cells *in vitro* in response to particles of prosthetic materials^{19,29}. Other, non-prostanoid mediators, including interleukin-1¹¹, tumor necrosis factor, and interleukin-6⁶, have been identified in the inflammatory response to wear particles. Prostaglandin E₂, interleukin-1, tumor necrosis factor, and interleukin-6 have all been implicated in the stimulation of bone resorption^{23,39}. The inflammatory effect of particles may be as harmful or more harmful than their toxic effect. Thus, we postulated that a less toxic particle that stimulates continuing synthesis and release of inflammatory mediators may be associated with more bone resorption. To examine this concept, we tested the hypothesis that particles of a metal alloy showing little toxicity would cause the release of greater amounts of inflammatory mediators than would a more toxic alloy. Wear particles of the commonly used titanium-aluminum-vanadium and cobalt-chromium alloys were chosen because the toxicity of cobalt-chromium-alloy particles has been documented *in vitro*⁹ and *in vivo*²¹, while there is evidence that titanium-aluminum-vanadium, in solid and particulate form, is relatively non-toxic^{33,34}.

We examined the effects of these particles on macrophages, as we believe that macrophages play a central role in the response to wear particles. Macrophages are known to be producers of a large number of potent inflammatory mediators, many of which stimulate bone resorption⁷. Large numbers of these cells have also been found at sites of excessive production of wear particles associated with failed prostheses⁴⁰. In addition, long-term *in vivo* animal studies have shown a strong and persistent macrophage response to metal-alloy particles^{20,21,40}.

Many of the problems in the interpretation of the results of previous comparative studies of the effects of wear particles have been due either to the relatively poor sensitivity and precision of *in vivo* tests or to differences in the sizes or numbers of the particles of material that were tested. To avoid these obstacles, we used *in vitro* assays, which allow quantitative measurement of the effects of particles; we also used particles of each material that were of comparable size and concentration and were of a concentration similar to that which has been found in the tissues around failed prostheses.

The principal aim of this study was to test the hypothesis that titanium-aluminum-vanadium particles, while less toxic than cobalt-chromium particles, induce the release of more prostaglandin E₂. We also examined the differences in the release of the inflammatory cytokines interleukin-1, tumor necrosis factor, and

interleukin-6 and determined the patterns of toxicity and mediator release in response to increased concentrations of titanium-aluminum-vanadium and cobalt-chromium wear particles.

Materials and Methods

Generation of Wear Particles

Hollow metal titanium-aluminum-vanadium and cobalt-chromium-alloy chambers, with blocks of the same metal alloy sealed inside, were shaken with use of a flask shaker (Gallenkamp, Loughborough, United Kingdom). The cobalt-chromium chamber (Howmedica, Rutherford, New Jersey) consisted of two femoral-head surface-replacement prostheses, containing ten Vitalium blocks that measured ten by five by five millimeters. To generate titanium-aluminum-vanadium-alloy particles, a titanium femoral head (Zimmer, Warsaw, Indiana) was halved and milled to produce two identical cups. Blocks, ten by four by four millimeters, from the same alloy, were cut from the stem of the same prosthesis. Before milling, the components were soaked in 20 per cent nitric acid for twenty minutes, which was followed by washing in distilled water according to the standards of the American Society for Testing and Materials² for the preparation of metallic implants and by sterilization with autoclaving. The cups containing blocks of the same metal and milling solution were clamped together, sealed with tape, and shaken for sixty hours. This solution was then discarded to eliminate contaminants from the metals. Next, the cups were shaken with fresh milling solution for forty-eight hours, after which the solution with metal particles was retrieved. The milling solution consisted of phosphate-buffered saline solution supplemented with 2 per cent rat serum, penicillin (five micrograms per milliliter), and streptomycin (fifty units per milliliter).

The recovered metal particles were sized by differential centrifugation according to methods described by Garrett et al.⁹, to obtain particles in the range of 0.5 to three micrometers²⁵. A sample of the isolated metal-particle suspension was analyzed with use of a Coulter multisizer (model 2; Coulter Electronics, Luton, United Kingdom) to confirm the size of the particles. The concentration of metal was determined with atomic absorption spectrometry. Metal content was determined with use of an inductively coupled plasma mass spectrometer (VG Plasmaquad PQ2 Plus; VG Isotopes, Winsford, United Kingdom). The relative levels of the cobalt, chromium, molybdenum, titanium, aluminum, vanadium, and nickel were measured, and the percentage composition was found to be similar to the manufacturer's specifications. The cobalt-chromium-alloy particles contained 58 per cent cobalt, 34 per cent chromium, 7.8 per cent molybdenum, and 0.2 per cent nickel. Titanium-aluminum-vanadium-alloy particles contained 89.3 per cent titanium, 7.2 per cent aluminum, and 3.5 per cent vanadium.

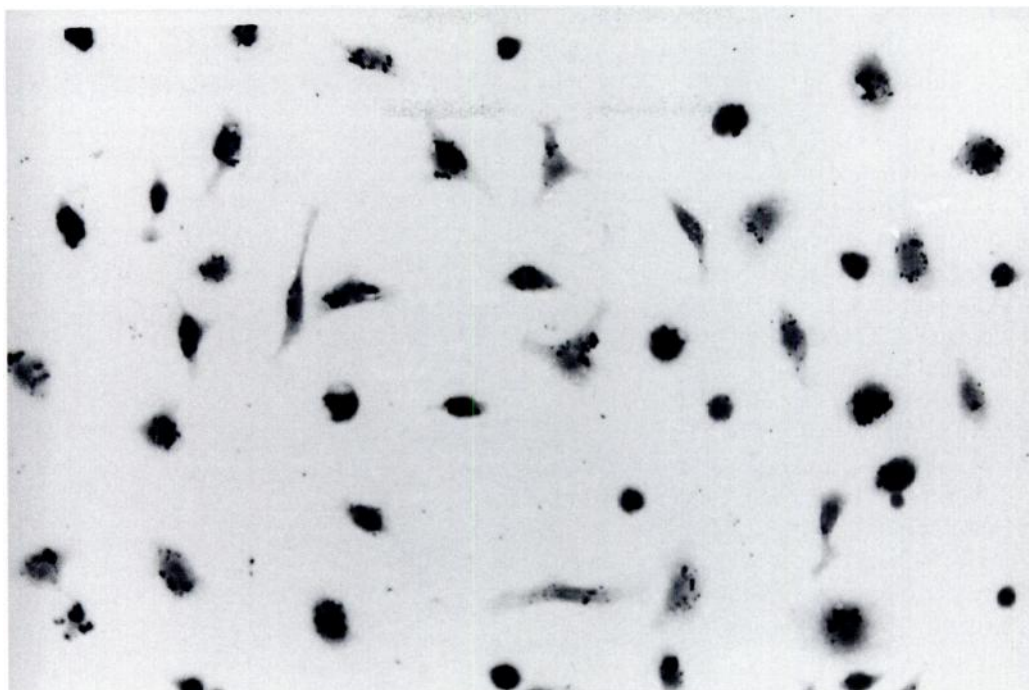


FIG. 1-A

Figs. 1-A and 1-B: Photomicrographs of cells that were cultured with metal particles for forty-eight hours, made after gentle washing was done to remove unbound and non-phagocytosed particles (Giemsa stain; original magnification $\times 300$).

Fig. 1-A: Cells cultured with titanium-aluminum-vanadium particles at a concentration of 2×10^6 per milliliter.

Isolation and Culture of Cells

Peritoneal macrophages from eight to twelve-week-old male dark agouti rats were isolated by adherence to glass, as described previously¹⁶, and incubated with various concentrations of the particles. Briefly, 1×10^6 peritoneal cells, suspended in one milliliter of RPMI-1640

medium with 10 per cent fetal calf serum, penicillin (five micrograms per milliliter), and streptomycin (fifty units per milliliter), were placed in sixteen-millimeter flat-bottomed wells of a twenty-four-well tray (Costar, Costa Mesa, California). After one hour of incubation at 37 degrees Celsius in 5 per cent carbon dioxide, the non-



FIG. 1-B

Cells cultured with cobalt-chromium particles at a concentration of 2×10^6 per milliliter.

adherent cells were removed by washing with Hanks balanced salt solution. The adherent cells were then incubated in one milliliter of RPMI medium containing the appropriate concentration of particles. Five micrograms per milliliter of lipopolysaccharide (*Escherichia coli* 0111:B4 [Sigma Chemical, St. Louis, Missouri]) was used as a strong stimulus to determine the ability of these cells to produce inflammatory mediators. After forty-eight hours, the supernatants were sampled and were centrifuged at 4000 times gravity for ten minutes to remove any particles. The samples were then tested for levels of inflammatory mediators either immediately or after storage at -70 degrees Celsius.

Toxic levels of particles may induce the release of mediators that are already present in the macrophages at the time of isolation by disrupting the cell membrane. The levels of these mediators were determined by the immediate lysing of isolated macrophages with 0.5 milliliter of sterile water, followed by restoration of the media to normal strength by the addition of 0.5 milliliter of double-strength media. We could not detect prostaglandin E_2 , interleukin-6, or tumor necrosis factor; however, 5.5 ± 0.4 (mean and standard error of six experiments) units per milliliter of interleukin-1 activity was present in these supernatants.

For photomicrography, non-phagocytosed and unbound particles were gently washed away before the cells were fixed in 2 per cent glutaraldehyde and were stained with Giemsa solution.

Assays for Toxicity

Trypan-blue exclusion¹⁵ and lactate-dehydrogenase release (Sigma Chemical)⁴¹ were both used to assess macrophage viability.

Assays for Mediators

A relatively specific cytotoxic interleukin-1 assay was carried out with use of the interleukin-1-sensitive A375 cell line, as described previously³⁰. Human recombinant interleukin-1- β , a gift from Otsuka Pharmaceutical (Tokushima, Japan), was used as a standard in these assays. Although these cells are also sensitive to high levels of tumor necrosis factor, the levels found in our supernatants were always at least ten times too low to be detected in the assays at the dilutions that were used.

A cytotoxic tumor necrosis-factor assay was performed with use of the tumor-necrosis-factor-sensitive cell line L929²⁸. Human recombinant tumor necrosis factor α (Genzyme, Boston, Massachusetts) was used as a standard in all assays. To assay interleukin-6, 7TD1-hybridoma cells were used as described previously⁴. Human recombinant interleukin-6 (Genzyme) was used as a standard in all of the assays.

In these cytokine bioassays, one unit per milliliter of activity was determined to be that present in a dilution of the sample that demonstrated 50 per cent of maximum activity. The appropriate recombinant cytokine was

TABLE I
COMPARISON OF THE EFFECTS OF METAL-ALLOY PARTICLES ON CELL VIABILITY AND ON THE RELEASE OF BONE-RESORBING MEDIATORS BY MACROPHAGES, AT CONCENTRATIONS AT WHICH COBALT-CHROMIUM PARTICLES EXHIBITED MILD AND MAXIMUM TOXICITY

	Cobalt-Chromium Particles	Titanium-Aluminum-Vanadium Particles	P Value*
Mild toxicity (1.8×10^6 particles of cobalt-chromium/ml)			
Cell viability (per cent)	85	95	0.001
Prostaglandin E_2 (nM/ml)	6.80	39.71	0.005
Interleukin-1 (units/ml)	5.5	6.0	0.267
Tumor necrosis factor (units/ml)	54.6	14.9	0.023
Interleukin-6 (units/ml)	2890	13,000	0.004
Maximum toxicity (6×10^7 particles of cobalt-chromium/ml)			
Cell viability (per cent)	17	88	<0.0001
Prostaglandin E_2 (nM/ml)	0.70	49.0	0.002
Interleukin-1 (units/ml)	4.6	56.9	0.004
Tumor necrosis factor (units/ml)	26.2	135	0.050
Interleukin-6 (units/ml)	1710	31,700	0.005

*A p value of less than 0.013 was considered significant when the hypothesis was tested (see text).

included in each assay to monitor variations between assays that had been carried out at different times. Supernatants from media that had been incubated for forty-eight hours with cobalt-chromium or titanium-aluminum-vanadium particles in the absence of cells did not affect these biological assays at the concentrations used to measure activity, except for interleukin-1 (as will be discussed in the Results section).

The level of prostaglandin E_2 was determined with use of a competitive radioimmunoassay²⁴. 3H -labeled prostaglandin E_2 was purchased from Amersham (Buckinghamshire, United Kingdom), and unlabeled prostaglandin E_2 was obtained from Sigma Chemical.

Study Design and Statistical Analysis

To test our hypothesis, we first needed to determine if there were significant differences in toxicity between the titanium-aluminum-vanadium and cobalt-chromium particles and, if there were, to compare the levels of prostaglandin- E_2 release. This was done at two concentrations of cobalt-chromium particles: at the lowest concentration that induced mild toxicity (more than a 10 per cent reduction in viability) and at the concentration that induced maximum toxicity. The significance of the difference in the release of prostaglandin E_2 at these two

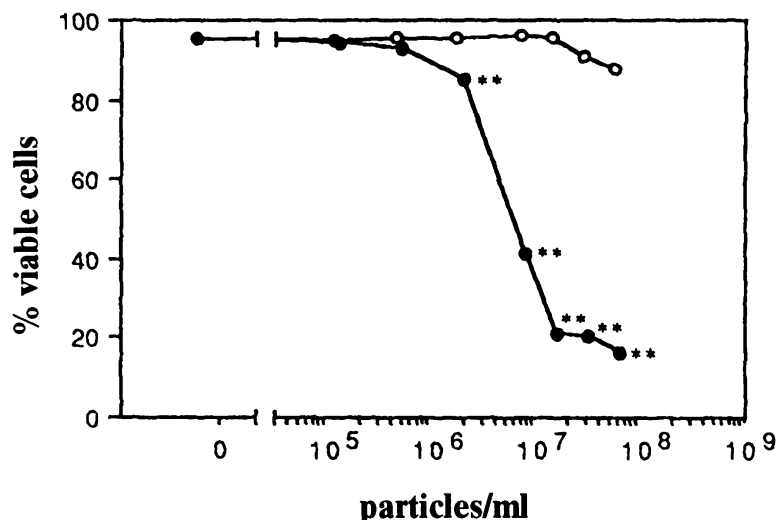


FIG. 2

Graph showing the effect of titanium-aluminum-vanadium (open circles) and cobalt-chromium particles (solid circles) on the viability of peritoneal macrophages in rats. The percentage of viable cells was assessed by trypan-blue exclusion. Each point represents the mean of at least nine experiments. The differences between the results of the metal particle cultures and the control values (no particles) were analyzed with a paired Student t test. Two asterisks = $p < 0.005$.

concentrations was then determined. Next, the differences in the release of interleukin-1, tumor necrosis factor, and interleukin-6 were determined for both types of particles at the same two concentrations. In addition, the patterns of toxicity and release of all of the mediators were determined for a wide range of particle concentrations. Statistical significance was calculated with use of a paired Student t test³⁷. To adjust for the experiment-wise chance of a spurious significant difference, we established our hypothesis and did four tests (of the four bone-resorbing mediators). We chose a p value of less than 0.013 instead of 0.05 to make a correction for the multiple comparisons when testing the hypothesis, as has been previously recommended⁸. This comparison

was not made for the rest of the study, as it involved differences observed outside of the testing of the hypothesis (Figs. 2 through 6), for which a p value of less than 0.05 was considered appropriate.

Results

Both types of particles were readily phagocytosed by macrophages. Exposure to titanium-aluminum-vanadium particles did not affect the morphology of the macrophages, but the ingestion of cobalt-chromium particles caused most of the cells to take on a more rounded shape (Figs. 1-A and 1-B).

There were clear differences in the toxicity and release of inflammatory mediators between the two types

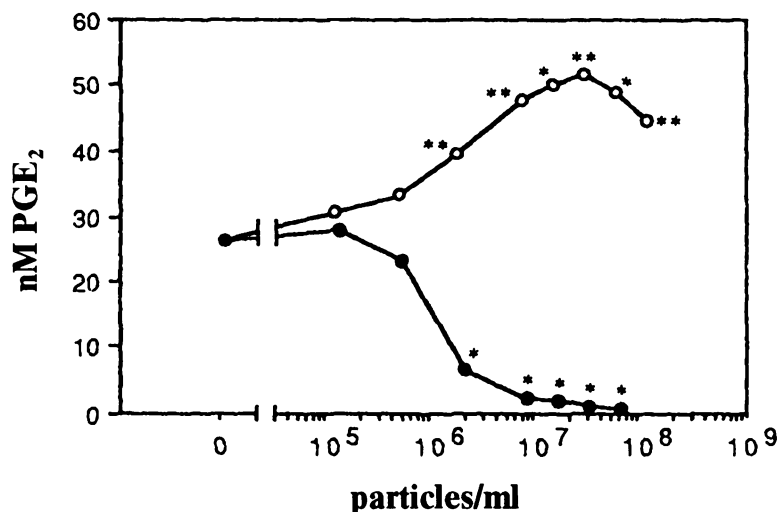


FIG. 3

Graph showing the effect of titanium-aluminum-vanadium (open circles) and cobalt-chromium particles (solid circles) on the release of prostaglandin E₂. Each point represents the mean of at least nine experiments. The differences between the results of the metal particle cultures and the control values (no particles) were analyzed with a paired Student t test. One asterisk = $p < 0.05$ and two asterisks = $p < 0.005$.

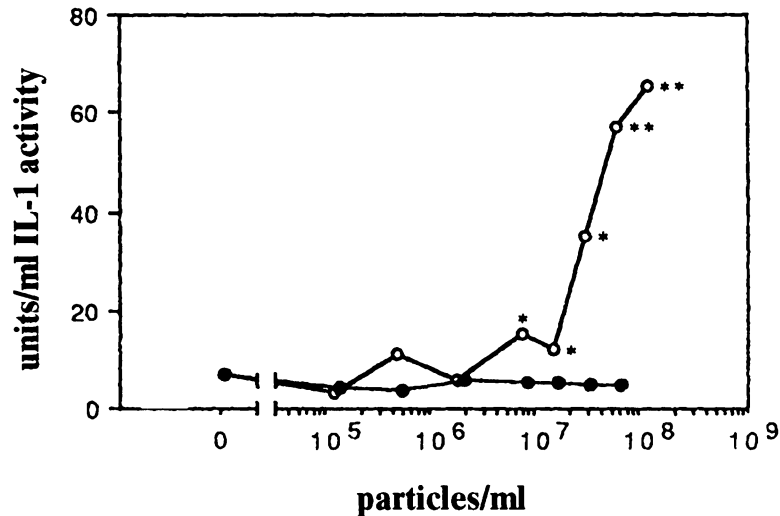


FIG. 4

Graph showing the effect of titanium-aluminum-vanadium (open circles) and cobalt-chromium particles (solid circles) on the release of interleukin-1. Each point represents the mean of at least nine experiments. The differences between the results of the metal particle cultures and the control values (no particles) were analyzed with a paired Student t test. One asterisk = $p < 0.05$ and two asterisks = $p < 0.005$.

of metal particles (Table I). The lowest concentration of cobalt-chromium particles that induced mild toxicity was 1.8×10^6 particles per milliliter. At this concentration, cobalt-chromium particles were more toxic than titanium-aluminum-vanadium particles. However, approximately six times more prostaglandin E_2 was released with exposure to titanium-aluminum-vanadium particles than with exposure to cobalt-chromium particles ($p = 0.005$ [Table I]).

The concentration of cobalt-chromium that induced maximum toxicity was 6×10^7 particles per milliliter. At this concentration, cobalt-chromium particles were far more toxic than titanium-aluminum-vanadium particles, which induced a seventyfold increase in the release of prostaglandin E_2 ($p = 0.002$ [Table I]). These differences were significant, and our hypothesis was confirmed.

At the concentration of cobalt-chromium particles that was mildly toxic, exposure to titanium-aluminum-vanadium particles induced release of similar levels of interleukin-1 ($p = 0.267$), more than threefold less tumor necrosis factor ($p = 0.023$), and nearly fivefold more interleukin-6 ($p = 0.004$) compared with the levels released by exposure to cobalt-chromium particles (Table I). At the concentration of cobalt-chromium particles that caused maximum toxicity, exposure to titanium-aluminum-vanadium particles induced release of much larger amounts of interleukin-1 ($p = 0.004$), tumor necrosis factor ($p = 0.050$), and interleukin-6 ($p = 0.005$) (Table I).

There were differences in the patterns of toxicity between the two types of particles (Fig. 2). Exposure to cobalt-chromium particles induced toxicity at a wide range of concentrations, beginning at 10^6 particles per milliliter. These particles were mildly toxic at concentrations of 2×10^6 particles per milliliter, and a marked reduction in cell viability was seen at higher concen-

trations. In contrast, exposure to titanium-aluminum-vanadium particles, even at high concentrations, had little effect on the percentage of viable cells. Similar data were obtained at all concentrations of particles when the cell viability was assessed either with the trypan-blue exclusion method or with measurement of lactate-dehydrogenase release into the supernatant. The trypan-blue exclusion method was considered unreliable for this assessment at concentrations of 1×10^8 particles per milliliter, as the numbers of particles that had phagocytosed made it difficult to determine nuclear staining. However, measurement of the release of lactate dehydrogenase indicated that these cells remained viable at concentrations of 1×10^8 particles of titanium-aluminum-vanadium per milliliter.

Exposure to titanium-aluminum-vanadium particles stimulated prostaglandin- E_2 release compared with control values at concentrations of 1.8×10^6 particles per milliliter or more (Fig. 3). The highest levels were observed at concentrations of 10^7 to 10^8 particles per milliliter. These levels were about half those seen after lipopolysaccharide stimulation (111 nanomoles per milliliter). At levels of more than 2×10^6 particles per milliliter, exposure to cobalt-chromium particles did not stimulate the prostaglandin- E_2 release but, rather, reduced it.

Interleukin-1 activity in the supernatants was elevated by exposure to titanium-aluminum-vanadium particles at concentrations of 8×10^6 particles per milliliter or more (Fig. 4). At concentrations of titanium-aluminum-vanadium that approached 10^8 particles per milliliter, there was a tenfold increase above baseline. These levels were even higher than those released after lipopolysaccharide stimulation (29.5 units per milliliter). The media that had been incubated with 10^7 to 10^8 particles per milliliter of titanium-aluminum-vanadium

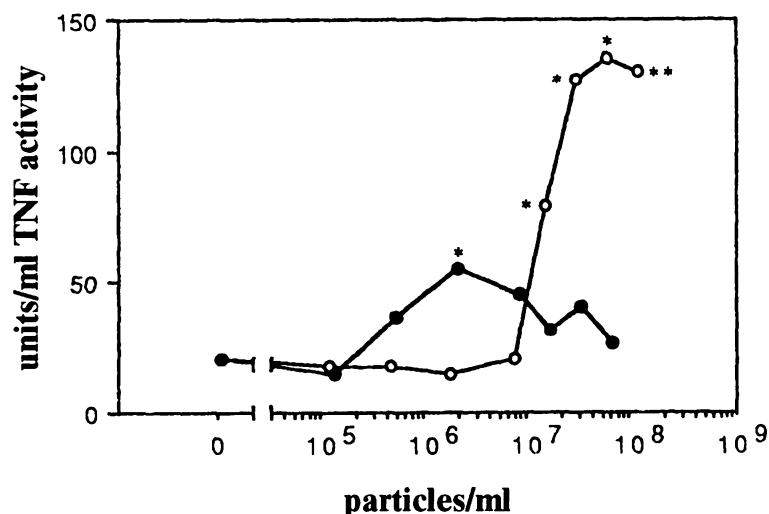


FIG. 5

Graph showing the effect of titanium-aluminum-vanadium (open circles) and cobalt-chromium particles (solid circles) on the release of tumor necrosis factor. Each point represents the mean of at least nine experiments. The differences between the results of the metal particle cultures and the control values (no particles) were analyzed with a paired Student *t* test. One asterisk = $p < 0.05$ and two asterisks = $p < 0.005$.

in the absence of cells for forty-eight hours had detectable interleukin-1-like activity, as indicated by inhibition of proliferation of A375 cells. However, these levels were less than one-fifth of the activity obtained by the stimulation of macrophages with exposure to the same concentrations of titanium-aluminum-vanadium particles.

Exposure to titanium-aluminum-vanadium particles stimulated release of tumor necrosis factor in a pattern similar to that of interleukin-1 (Fig. 5). Exposure to concentrations of 10^7 particles per milliliter or more resulted in marked increases in the activity of tumor necrosis factor in the supernatant. At concentrations approaching 10^8 particles per milliliter, the levels of tumor necrosis factor were about sevenfold higher than

those released from resting cells. These levels were approximately the same as those seen after lipopolysaccharide stimulation (144 units per milliliter). Exposure to cobalt-chromium particles induced a slight increase in the release of tumor necrosis factor at concentrations of 1.8×10^6 particles per milliliter.

Exposure to titanium-aluminum-vanadium stimulated interleukin-6 activity, but exposure to cobalt-chromium particles did not (Fig. 6). As with interleukin-1 and tumor necrosis factor, there was a marked increase in the release of interleukin-6 at concentrations of 8×10^6 particles per milliliter or more. However, the approximately threefold increase in interleukin-6 activity was lower than that seen after lipopolysaccharide stimulation (77,800 units per milliliter). Exposure to cobalt-

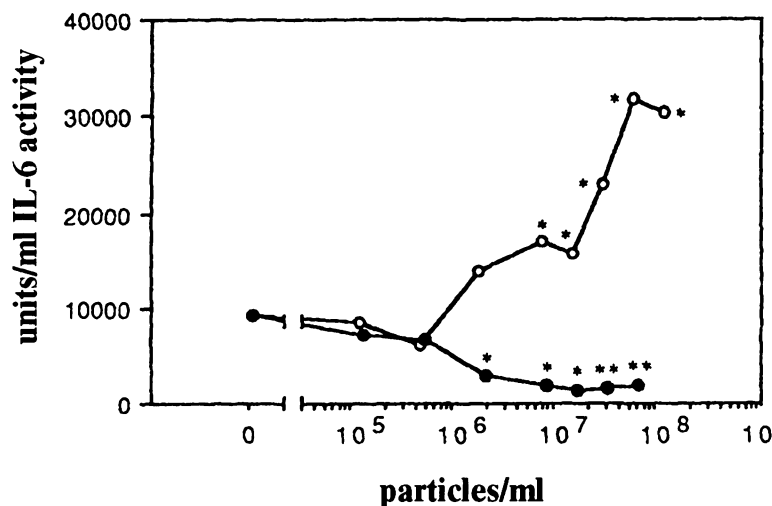


FIG. 6

The effect of titanium-aluminum-vanadium (open circles) and cobalt-chromium particles (solid circles) on the release of interleukin-6. Each point represents the mean of at least nine experiments. The differences between the results of the metal particle cultures and the control values (no particles) were analyzed with a paired Student *t* test. One asterisk = $p < 0.05$ and two asterisks = $p < 0.005$.

chromium reduced interleukin-6 levels at concentrations of 2×10^6 particles per milliliter or more.

Discussion

The results support our hypothesis that titanium-aluminum-vanadium wear particles are less toxic than cobalt-chromium wear particles but they induce release of more prostaglandin E_2 , which has been implicated in bone resorption and loosening of prostheses. At concentrations at which cobalt-chromium particles were mildly and maximally toxic to macrophages, much higher levels of prostaglandin E_2 were released by macrophages that had been exposed to titanium-aluminum-vanadium particles than by those that had been exposed to cobalt-chromium particles. This strongly supports the contention that, although wear particles may cause cell death, resulting in the release of cytoplasmic or cell-surface factors, considerably more release of inflammatory mediators may be caused by stimulation of cells that remain viable. Although large differences between the responses to the two types of particles were seen at the end of the forty-eight hours of *in vitro* testing, these differences may be even greater *in vivo* since the non-toxic particles may continue to stimulate the release of these factors over a long period of time. Prostaglandin E_2 and interleukin-1 are considered to be major inducers of bone resorption, and we believe that the strong stimulation of prostaglandin E_2 by the less toxic titanium-aluminum-vanadium particles is of considerable importance.

The study also showed that release of interleukin-1 was stimulated by exposure to titanium-aluminum-vanadium but not by exposure to cobalt-chromium particles at either mildly or maximally toxic concentrations. Interestingly, the release of tumor necrosis factor was greater from the cells that had been exposed to cobalt-chromium particles at mildly toxic concentrations (Table I). The release of interleukin-6, similar to that of prostaglandin E_2 , was greater after exposure to titanium-aluminum-vanadium particles.

Our findings support the findings of previous *in vivo* studies that have suggested that cobalt-chromium-alloy particles are more toxic than titanium-aluminum-vanadium-alloy particles. The intra-articular injection of cobalt-chromium wear particles has caused cell toxicity²¹, while titanium-aluminum-vanadium particles have been found to be relatively non-toxic²². *In vitro* studies have demonstrated the importance of cobalt-chromium particles in the inducement of macrophage death⁹. It is interesting that, while vanadium may be toxic to cells, its presence in the titanium-aluminum-vanadium particles does not make these particles particularly toxic compared with cobalt-chromium particles³³.

In order to reproduce *in vivo* conditions as closely as possible, we assessed the effect of exposure to metal particles of a size, shape, and concentration similar to those reported around failed prostheses in humans. With

regard to size, studies involving light microscopic and electron microscopic examination of specimens of periprosthetic tissues^{26,40} and isolation of particles from these tissues²⁶ have shown most metal particles to be in the size-range of 0.5 to two micrometers. Analysis of the particles with a Coulter multisizer in the current study showed that nearly all of the particles were within this range, with a mean size of about one micrometer, and that the distribution of particles was very similar to distributions that have been reported *in vivo*²⁶. The particles of cobalt-chromium and titanium-aluminum-vanadium that were produced by our methods were often the shape of short needles with jagged edges, resembling those that have been observed *in vivo*²⁶.

We used concentrations of particles similar to those that have been found in human periprosthetic tissues. With an assumption of an average particle size of one micrometer, concentrations as high as 5×10^7 and 2.5×10^6 particles per gram or milliliter of tissue have been reported for titanium-aluminum-vanadium and cobalt-chromium, respectively^{1,22}. We found that similar and fewer numbers of particles per milliliter reduced the viability of cells exposed to cobalt-chromium and stimulated release of mediators in those exposed to titanium-aluminum-vanadium *in vitro*. Examination of the tissue surrounding failed joint prostheses^{1,20,40} has often revealed macrophages containing metal particles very similar in shape and size to those that we observed *in vitro* (Figs. 1-A and 1-B).

Cytokine production was assessed with use of biological assays, which allow measurement of the sum of the actions of the cytokine that is produced and of any inhibitors that are also present in the test sample. We were careful to select assays that are relatively specific and not affected by other factors, such as prostaglandins, which inhibit the other interleukin-1 biological assays. We cannot rule out the effects of other factors, such as specific inhibitors¹⁴, that might also be regulated by the particles present in the supernatants. However, it is the total biological activity — that of the cytokine and that of any inhibitors — induced by the particles that will determine the effects on tissues, including activities such as bone resorption.

The implications of cobalt-chromium particles being more toxic to macrophages than titanium-aluminum-vanadium particles are interesting. Since both types of particles were of the same size and of similar shape, it seems likely that the differences in toxicity are due to the chemical properties of the metal alloys. Disruption of the cells by toxic levels of cobalt-chromium may induce release of interleukin-1 associated with the cell¹⁰. Therefore, even though new synthesis of interleukin-1 may have been inhibited by the toxic effects of cobalt-chromium particles, the over-all levels in the supernatant may have been compensated for by the release of interleukin-1 that was present in the macrophages at the time of isolation. High levels (more than ten units per

milliliter) of interleukin-1, but not of tumor necrosis factor, interleukin-6, or prostaglandin E₂, were detected in the supernatant of cells that had been lysed immediately with water, indicating that interleukin-1 was the only mediator already present in these macrophages at marked levels. This shows that, although toxic wear particles may inhibit new production of mediators, pre-formed mediators can be released due to subsequent cell damage. However, in the current study, only relatively small amounts of pre-formed interleukin-1 were released in this way compared with the amounts released by titanium-aluminum-vanadium particles at concentrations greater than 2×10^7 .

The levels of mediator release were calculated as units of activity of cytokine or of nanomolar prostaglandin E₂ per milliliter of supernatant. These levels could have been represented for each viable cell. When the effects of exposure to titanium-aluminum-vanadium and cobalt-chromium particles were compared in this way, there were still large and statistically significant differences between the two types of particles with regard to the release of prostaglandin E₂ and interleukin-6, at a wide range of concentrations. However, the calculation of mediator levels for each viable cell may not be appropriate, since cobalt-chromium particles can take several hours to induce cell death. Our own unpublished time-course studies have shown that this may take as long as twenty-four hours. Cells that have been identified as non-viable at the end of the experiment (forty-eight hours) may have produced significant amounts of mediators before cell death had occurred. For example, a significant increase in the release of tumor necrosis factor was observed at toxic concentrations of cobalt-chromium (10^6 to 10^7 particles per milliliter). This may indicate that tumor necrosis factor, which is known to be released within several hours after cell stimulation⁷, was formed and released before the cells were killed.

Our assessments of cell viability were based on the

access of trypan blue as well as the movement of lactate dehydrogenase through the cell membrane. Other cell functions may be affected at concentrations lower than those that disrupt the cell membrane, and consequently, we may have underestimated the toxicity of these particles.

Significant inhibition of prostaglandin E₂ and interleukin-1 was observed at a concentration of particles that reduced cell viability by less than 10 per cent. This may indicate that cell functions essential for the production and release of these mediators are more sensitive to the toxic effects of cobalt-chromium particles than is the cell membrane.

Several of the mediators tested in our study can either stimulate⁷ or inhibit¹⁷ both their own release and that of other mediators involved in bone resorption. For example, prostaglandin E₂ is a strong stimulator of interleukin-6 production in monocytes and macrophages¹⁷, and it is possible that titanium-aluminum-vanadium particles may first stimulate production of prostaglandin E₂, which in turn may induce release of interleukin-6. Conversely, prostaglandin E₂ can also inhibit release of tumor necrosis factor and, possibly, production of interleukin-1^{17,36}. The stimulation of prostaglandin-E₂ release by metal particles that we and others have observed¹² may play an important role in the regulation of other biological mediators of bone resorption.

In summary, exposure to cobalt-chromium particles was found to be very toxic to macrophages. In contrast, titanium-aluminum-vanadium particles were only minimally toxic, but they induced high levels of mediator release at a wide range of concentrations. We believe that this type of response indicates that the generation of wear particles, such as those from titanium-aluminum-vanadium implants, that are only minimally toxic but that stimulate mediators involved in bone resorption is potentially detrimental to the fixation of prostheses.

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