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The immune dysregulatory compound mercuric chloride induces integrin-mediated T-lymphocyte adhesion

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SUMMARY

Exposure of Brown Norway rats to mercuric chloride induces systemic autoimmunity, involving T- and B-lymphocyte activation, (auto-)antibody production and multiorgan inflammation. Several divalent metal ions, such as Mg^{2+} and Mn^{2+}, can activate binding of integrins to their ligands, thus causing lymphocyte adhesion. To test the hypothesis that Hg^{2+} acts in a similar way, we studied the effect of $HgCl_2$ on integrin-mediated T-cell adhesion. $HgCl_2$ induced cell–cell aggregation of human T lymphoblasts. Exposure of a human T-cell clone to $HgCl_2$ for 1 hr enhanced, in a dose-dependent way, cell binding to fibronectin (FN) and to intercellular adhesion molecules (ICAM) -1, -2 and -3. Furthermore, $HgCl_2$ induced strong binding of Jurkat T cells to FN. These effects of $HgCl_2$ were of similar magnitude as the effects of phorbol 12-myristate 13-acetate (PMA) or $MnCl_2$. Studies using blocking antibodies indicated the involvement of CD11a in binding to ICAMs, and of CD49d, CD49e, and CD29 in binding to FN. Adhesion to FN induced by $HgCl_2$ or by PMA, but not by $MnCl_2$, was dependent on temperature and on extracellular Ca^{2+} or Mg^{2+}. Addition of cytochalasin B enhanced synergistically the FN adhesion induced by $MnCl_2$, whereas the effects of PMA and $HgCl_2$ were not modified. These results indicate that Hg^{2+} is a potent activator of T-cell adhesion, mediated by several integrins and ligands. In contrast to the effect of $MnCl_2$, $HgCl_2$-induced cell adhesion probably involves an intracellular pathway. Activation of integrins by $HgCl_2$ may play an important role in activation and migration of leucocytes involved in $HgCl_2$-induced immune dysregulation *in vivo*.

INTRODUCTION

A number of drugs and environmental pollutants can induce immune dysregulation in susceptible individuals, which may for example lead to autoimmune diseases involving the kidney as a target organ.[1] Using mercuric chloride as a model compound, immune dysregulation induced by chemicals has been frequently studied in rodents. Exposure of Brown Norway (BN) rats to $HgCl_2$ induces a systemic lupus-like autoimmune syndrome, characterized by activation and proliferation of T and B lymphocytes, high production of (auto-) antibodies, infiltration of leucocytes in many organs, glomerulonephritis and proteinuria.[2,3] T-cell depletion experiments revealed an essential role for T lymphocytes in disease induction.[4] Furthermore, high production of interleukin-4 (IL-4) and immunoglobulin E (IgE) in $HgCl_2$-exposed BN rats indicate a strong and preferential activation of T helper type 2 (Th2) cells.[5,6] The mechanism of T-cell activation by exposure to $HgCl_2$ has not been identified in detail. $HgCl_2$ may alter the structure, processing, presentation and/or recognition of (auto-)antigens, thus leading to T-cell autoreactivity.[7] In addition, non-antigen-specific T-lymphocyte activation by $HgCl_2$ is likely to be involved.[8,9]

We have previously shown that *in vivo* exposure of BN rats to $HgCl_2$ enhances T-lymphocyte expression of various cell adhesion and activation molecules, such as lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18), intercellular adhesion molecule-1 (ICAM-1; CD54), integrin α_4 (CD49d) and OX40 (CD134), at an early stage of disease.[10] Furthermore, it has been reported by Molina *et al*. that *in vivo* treatment with a blocking monoclonal antibody (mAb) directed against integrin α_4 inhibits a major part of autoimmune

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Abbreviations: FN, fibronectin; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; mAb, monoclonal antibody; VLA, very late antigen.

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manifestations induced by $HgCl_2$ in BN rats.[11] These data indicate a role for cell adhesion molecules in the immune dysregulation induced by $HgCl_2$. In the present study, we examined whether $HgCl_2$ may have a direct effect on T-cell adhesion mediated by integrins.

Integrins are heterodimeric cell membrane receptors involved in cell-to-cell and cell-to-matrix adhesion. Integrin-mediated adhesive capacity is determined by the levels of expression of the receptors and their ligands.[12] Furthermore, the receptors require activation for ligand binding.[13] Ligand binding results in cell adhesion, as well as in signal transduction via the integrin receptor,[14] and, in several cases, via the ligand.[15] Thus, these processes play an essential role in numerous functions of lymphocytes.

Activation of integrins can take place either as a result of extracellular events, or via an intracellular pathway. Certain integrin-binding mAbs can stimulate ligand binding, probably by stabilizing an active conformation of the receptor.[16–18] Furthermore, several divalent metal ions that bind to extracellular domains of integrins, such as Mg^{2+} and Mn^{2+}, increase integrin affinity for its ligand,[19,20] which can also be demonstrated using isolated integrins and ligands in a cell-free system, indicating its independence on cell function.[21] In contrast, integrin activation can also result from a process named inside-out signalling. This can be achieved by ligation of cell surface molecules, such as CD3 and CD2,[22,23] as well as by agents that induce intracellular signalling events, such as phorbol esters, which activate protein kinase C, and ionomycin, which elevates the intracellular calcium level.[24] Evidence has been presented that integrin activation by the phorbol ester phorbol 12-myristate 13-acetate (PMA) can take place independent of changes in integrin affinity, but involves modification of the association of integrins with cytoskeletal proteins, and their mobility in the plasma membrane.[20,25–27]

Induction of lymphocyte adhesion by $HgCl_2$, thus modifying the physiological regulation of this process, could be of direct importance for the immune dysregulatory capacity of this compound. In the present study, we investigated the effect of exposure of T lymphocytes to $HgCl_2$ on their binding to several ligands of integrins: fibronectin (FN), ICAM-1, ICAM-2 and ICAM-3. The effects of $HgCl_2$ were compared to adhesion induced by $MnCl_2$ and by PMA. The results show that $HgCl_2$ induces integrin-mediated T-cell adhesion, which involves several integrins and ligands and is probably mediated via an intracellular pathway.

MATERIALS AND METHODS

Cells and cell culture

Human peripheral blood obtained from healthy donors was used for isolation of peripheral blood mononuclear cells (PBMC) by density gradient centrifugation, using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). T lymphoblasts were generated by culturing PBMC for 5–7 days in the presence of soluble OKT3 (mAb anti-CD3) and of a cytokine mixture, that was obtained from activated PBMC, as described,[28] using RPMI culture medium (RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamin). The human Th0 cell clone ACC6 (kindly provided by Dr M. L. Kapsenberg, Amsterdam, the Netherlands), was stimulated every 14 days by allogeneic feeder cells and phytohaemagglutinin, as described,[29] and cultured in Iscove's modified Dulbecco's medium (Gibco BRL Life Technologies, Paisley, UK), supplemented with 10% heat-inactivated normal human serum, 25 U/ml recombinant IL-2 (Eurocetus, Amsterdam, the Netherlands), 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamin. ACC6 T cells were used for experiments when they were in a resting phase, at day 10 or 11 after stimulation. The T-cell leukaemia line Jurkat [obtained from the American Type Tissue Collection (ATCC), Rockville, MD] was cultured in RPMI culture medium.

Monoclonal antibodies

Mouse mAb were used, directed against CD3 (OKT3, IgG2a; supernatant from hybridoma obtained from the ATCC), CD11a (F8.8,[30] IgG1; kindly provided by Dr A. Bloem, Utrecht, the Netherlands, and LFA 1/2,[14] IgG1; donation from Dr R. A. W. van Lier, Amsterdam, The Netherlands), CD29 (4B4,[31] IgG1; Coulter Immunology, Hialeah, FL), CD49d (HP2/1,[11,32] IgG1; a gift from Dr F. Sánchez-Madrid, Madrid, Spain), CD49e (SAM-1, IgG2b; provided by Dr C. G. Figdor, Nijmegen, the Netherlands) and CD54 (84H10,[33] IgG1; from Dr S. Shaw, Bethesda, MD). Monoclonal mouse IgG1 (Zymed Laboratories, San Francisco, CA) was applied as a non-binding control mAb. All antibody preparations used were free of NaN_3.

Homotypic aggregation assay

Cells were cultured in flat-bottom 96-well plates (2×10^5/100 μl/well) at 37°, using adhesion buffer [Hanks' balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} (Gibco BRL), supplemented with D-glucose (2 mg/ml), bovine serum albumin (BSA; 10 mg/ml), $MgCl_2$ (1 mM) and $CaCl_2$ (1 mM)], in the presence or absence of $HgCl_2$ (Sigma, St Louis, MO). Monoclonal antibodies were preincubated with the cells during 30 min at 4°. Cell aggregation was examined by light microscopy after 3–4 hr of culture.

Production of chimeric proteins

Four different chimeric proteins containing the extracellular domains of either ICAM-1, ICAM-2, ICAM-3, or CD8, linked to the hinge domain of human IgG1, were expressed in COS cells. The appropriate expression plasmids for ICAM-1-IgG and CD8-IgG were kindly provided by Dr B. Seed (Boston, MA) and Dr A. Aruffo (Seattle, WA), and have been described previously.[34,35] The cDNA sequences encoding the two or five amino-terminal immunoglobulin-like domains of ICAM-2,[36] or ICAM-3,[37] respectively, were amplified by polymerase chain reaction (PCR) using synthetic oligonucleotides and cDNA from U937 cells. Primer sequences were as follows: ICAM-2-sense, 5′ GCCCGGTCGACGCCGCCACCATGTCCTCT-TTCGGTTACAGGACCCTG (position 43–90); and antisense, 5′ GACTATGATGGGATCCTGGCTGTCCGACACAG-GCTCATAGATCTC (position 702–746);[36] ICAM-3-sense, 5′ CAGGTCGACGTAGCCATCGCCACCATGGTACCAT-CC (position 2–22); and antisense, 5′ CACGAAGACGGGGA-GATCTTGGGAGCTCCCAGCCTC (position 1439–1474).[37] Specificity of amplification products was verified by restriction site analysis. Subsequently, they were cloned into the CD8-IgG1-CDM8 vector,[35] followed by transfection into COS7m6 cells, as described.[37] Seven days after transfection, the supernatants were

harvested and stored at −80° until use. Immunoprecipitation and sodium dodecyl sulphate–polyacrylamide gel electrophoresis revealed production of recombinant proteins of the expected molecular weights; concentrations of chimeric proteins in culture supernatants were determined by enzyme-linked immunosorbent assay.

Cell adhesion assays

For coating of chimeric proteins, flat-bottom 96-wells plates were precoated using goat anti-human Fcγ (10 μg/ml in 50 mM Tris, pH 9·5; Jackson Immunoresearch Laboratories, West Grove, PA) for 1 hr at 37°. After blocking (4% BSA in PBS; 1 hr at 37°) and washing, diluted COS cell-supernatants containing chimeric proteins (0·2 μg/ml in PBS) were incubated for 1 hr at 37°. Human plasma fibronectin (10 μg/ml in PBS), kindly provided by Dr J. van Mourik (Amsterdam, the Netherlands), was coated for 2 hr at 37°, followed by a blocking step (4% BSA in PBS; 1 hr at 37°). All plates were washed with adhesion buffer before use.

Jurkat T cells or ACC6 T cells were washed in adhesion buffer, and added to the coated wells at $0{\cdot}8 \times 10^5$/well and $1{\cdot}25 \times 10^5$/well, respectively, followed by centrifugation for 5 min at 20 ***g*** and incubation for 1 hr at 37°, unless otherwise indicated. Subsequently, the plates were gently washed, using warm adhesion buffer, five to seven times, and stored without buffer at −80° until measurement.

Incubation of the cells was performed in the presence or absence of the following stimuli: $HgCl_2$ (2·5 μM), $MnCl_2$ (500 μM; Sigma) and PMA (50 ng/ml; Sigma); they were applied at the indicated concentrations, unless otherwise specified. In some experiments, soluble purified OKT3 was used (5 μg/ml). All mAb were preincubated with the cells for 30 min at 4°. For testing the dependence on extracellular cations, cells were washed and incubated in Ca^{2+}- and Mg^{2+}-free adhesion buffer containing BSA which had been previously extensively dialysed against Ca^{2+}- and Mg^{2+}-free HBSS. Some experiments were performed using cytochalasin B (5 μg/ml; Sigma), which was preincubated with the cells for 30 min at 37°; subsequently, it was present during the assay. Cytochalasin B was dissolved in dimethyl sulphoxide, which was added to the control cultures at equivalent concentration ($\leq$0·05% v/v).

Quantification of cell adhesion

Cells which were bound to the wells were quantified by a fluorometric assay, using the DNA-binding fluorochromes Hoechst 33258 and Syto 13 (both from Molecular Probes, Leiden, the Netherlands) for Jurkat T cells and ACC6 T cells, respectively. A cell lysis procedure was performed which was previously described by Rago *et al.*[38] Briefly, the plates were thawed at room temperature, and, after addition of 100 μl distilled water per well, were incubated at 37° for 1 hr. Subsequently, the plates were again frozen at −80°. After thawing, 100 μl TNE buffer (10 mM Tris, 2 M NaCl, 1 mM ethylenediaminetetraacetic acid, pH 7·4) containing Hoechst 33258 (32 μM) or Syto 13 (10 μM) was added, and fluorescence intensity was measured by a fluorometer (Perkin Elmer LS50), using appropriate wavelengths for excitation and emission (345/460 nm for Hoechst 33258 and 488/509 nm for Syto 13). The number of cells bound to each well was calculated by interpolation in a calibration line. Binding to the ligand and to the control-coating was assessed in parallel for each stimulus: ICAM-1, ICAM-2 and ICAM-3 were compared with CD8, and FN was compared with BSA. The percentage specific binding was calculated from triplicate wells using the following formula: $100 \times$[(number of cells bound to the ligand − number of cells bound to control coating)/number of input cells]. Results were summarized as the mean percentage specific binding to the ligand ± standard deviation.

RESULTS

Integrin-mediated cell adhesion induced by $HgCl_2$

In order to examine whether exposure to $HgCl_2$ modifies cell adhesion, its effect on lymphocyte aggregation was examined. Addition of 5 μM $HgCl_2$ to T lymphoblasts clearly increased the number and size of cell aggregates, as compared to the control culture (Fig. 1a,b). Cell aggregation in control cultures and in $HgCl_2$-stimulated cultures was completely inhibited when the cells were preincubated with mAb against CD11a and CD54, indicating the involvement of LFA-1 and ICAM-1 in this process (Fig. 1c,d). Exposure of the Th0 clone ACC6 to $HgCl_2$ also induced cell aggregation (not shown). In contrast, the human leukaemic T-cell line Jurkat did not aggregate upon exposure to $HgCl_2$, to PMA, or to $MnCl_2$.

Cell adhesion induced by $HgCl_2$ was further examined in quantitative ligand binding assays, in which the number of adhered cells was measured using DNA-binding fluorochromes. This method has the convenience and accuracy of a fluorometric assay, and excludes any possible interference of the detection method in the biological process, since the detecting agent is not present during the adhesion assay. As illustrated in Fig. 2, $HgCl_2$ enhanced the binding of ACC6 T cells to FN, ICAM-1, ICAM-2 and ICAM-3 dose-dependently, with a maximal effect at 0·6–1·25 μM. Cell binding to ICAM-1, ICAM-2 and ICAM-3 could be completely prevented by blocking antibodies directed against CD11a, irrespective of the stimulus used (not shown). Exposure of Jurkat T cells to $HgCl_2$ induced strong cell binding to FN, to a similar level as that induced by PMA or by $MnCl_2$, as shown in Fig. 3. Fibronectin adhesion of Jurkat T cells exposed to $HgCl_2$ was maximal at a concentration of 2·5 μM; this concentration did not induce any cytotoxicity as assessed by trypan blue exclusion and by microscopic evaluation of cell morphology. Notably, the effect of $HgCl_2$ was much stronger than the effect of $MnCl_2$, when compared at equimolar concentrations (2·5–5 μM; not shown). Analysis of the kinetics of $HgCl_2$-induced FN adhesion revealed maximal binding after 1 hr of stimulation, followed by a gradual decrease (not shown). In agreement with other investigators,[39] as well as with the lack of inducible cellular aggregation in Jurkat T cells, these cells did not bind to ICAM-2, neither under control conditions, nor after stimulation with $HgCl_2$, $MnCl_2$, or PMA.

Inhibition studies using blocking mAb were performed to examine which integrins are involved in $HgCl_2$-induced binding of Jurkat T cells to FN (Fig. 4). A mAb directed against the integrin β_1 subunit (CD29) completely blocked FN adhesion under control conditions, as well as after stimulation with $HgCl_2$, $MnCl_2$, or PMA. Monoclonal antibodies directed

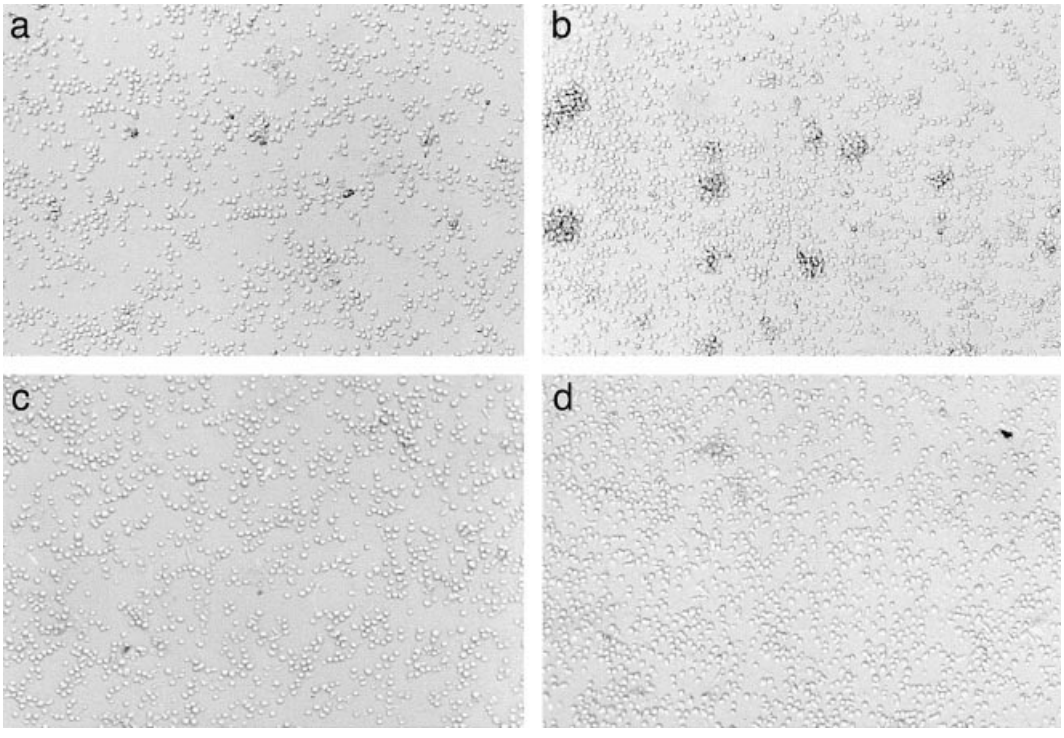

Figure 1. Lymphocyte aggregation induced by $HgCl_2$. T lymphoblasts, generated from human PBMC as explained in the Materials and Methods, were incubated in control medium (a, c), or in medium containing 5 μM $HgCl_2$ (b, d). Cells were preincubated in the presence (c, d) or absence (a, b) of LFA1/2 (anti-CD11a) and 84H10 (anti-CD54). Photographs were taken after 4 hr of incubation.

against integrin α_4 (CD49d) or α_5 (CD49e) subunits, when applied alone, did partially and variably inhibit FN adhesion. However, when they were applied in combination, FN adhesion was completely blocked, indicating that both integrin $\alpha_4\beta_1$ (VLA-4) and $\alpha_5\beta_1$ (VLA-5) were able to mediate cell adhesion to FN after stimulation with $HgCl_2$, $MnCl_2$, or PMA. Flow cytometric analysis revealed membrane expression of CD29, CD49d and CD49e on Jurkat T cells at a high level (not shown). Expression was not modified after incubation for 1 hr with $HgCl_2$, PMA, or $MnCl_2$, indicating that induction of cell adhesion by these stimuli is not caused by an increase in receptor number.

$HgCl_2$ and $MnCl_2$ induce cell adhesion via different pathways

Induction of Jurkat T-cell adhesion to FN by $HgCl_2$, $MnCl_2$, or PMA, was compared with respect to its dependence on extracellular cations and on a physiological temperature, in order to study the pathways used in the adhesion process.

When the adhesion assay was performed in a buffer without Ca^{2+} or Mg^{2+}, Jurkat T-cell adhesion to FN was completely blocked, unless $MnCl_2$ was present (Fig. 5a). Addition of 1 mM $CaCl_2$ (Fig. 5a) or 1 mM $MgCl_2$ (Fig. 5b) restored the effects of $HgCl_2$ and PMA but did not significantly modify the effect of $MnCl_2$. The presence of $MgCl_2$ (1 mM) also enhanced adhesion of untreated cells (Fig. 5); when $MgCl_2$ was applied at higher concentrations (3 mM), adhesion of unstimulated cells was further increased, and the stimulatory effects of $HgCl_2$, PMA and $MnCl_2$ were undetectable (not shown). These experiments indicate that Mn^{2+}, but not Hg^{2+}, bypasses the requirement of extracellular Ca^{2+} or Mg^{2+} for integrin-mediated cell adhesion.

Cell adhesion induced by $HgCl_2$ or by PMA was completely prevented when the assay was performed at 4° instead of at 37° whereas the effect of $MnCl_2$ was unaffected by incubation at low temperature (similar data were obtained in three independent experiments; not shown). Apparently, an active cellular process is required for adhesion induced by $HgCl_2$ or by PMA but, as expected, not for $MnCl_2$-induced adhesion.

Involvement of the actin cytoskeleton in cell adhesion

The compound cytochalasin B, which disrupts the intracellular actin cytoskeleton, was used to investigate the role of the cytoskeleton in FN adhesion of Jurkat T cells, induced by different stimuli. Pre-incubation with cytochalasin B induced a dose-dependent increase of adhesion of Jurkat T cells to FN (not shown). Furthermore, treatment with cytochalasin B had a synergistic effect when combined with $MnCl_2$, also when $MnCl_2$ was applied at a dose which was in itself not effective in induction of cell adhesion (Fig. 6). However, no effect of cytochalasin B was detected when it was applied in combination with PMA or $HgCl_2$ (Fig. 6).

DISCUSSION

The present study identifies the immune dysregulatory compound $HgCl_2$ as a potent activator of integrin-mediated T-cell adhesion. This effect of $HgCl_2$ is not specific for a single integrin or ligand: $HgCl_2$ activates cell binding via LFA-1 to

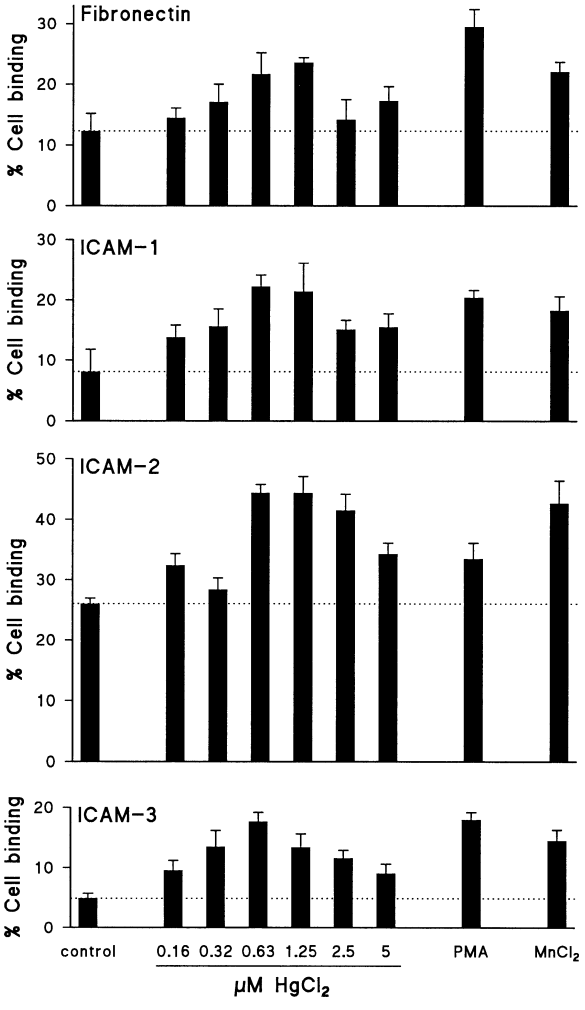

Figure 2. $HgCl_2$ induces binding of the Th0 clone ACC6 to FN, ICAM-1, ICAM-2 and ICAM-3. Adhesion of ACC6 T cells was assessed without stimulation (control) or in the presence of $HgCl_2$ (concentrations as indicated), PMA (50 ng/ml) or $MnCl_2$ (500 μM). One of two similar experiments is shown.

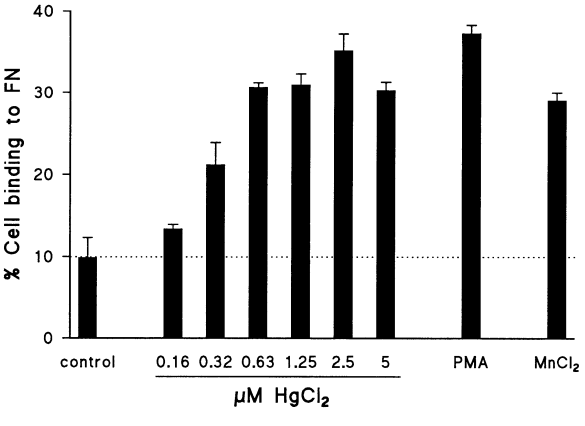

Figure 3. $HgCl_2$ induces adhesion of Jurkat T cells to FN. Jurkat T-cell adhesion was analysed without stimulation (control) or in the presence of $HgCl_2$ (concentrations as indicated), PMA (50 ng/ml) or $MnCl_2$ (500 μM). Data are representative for four experiments.

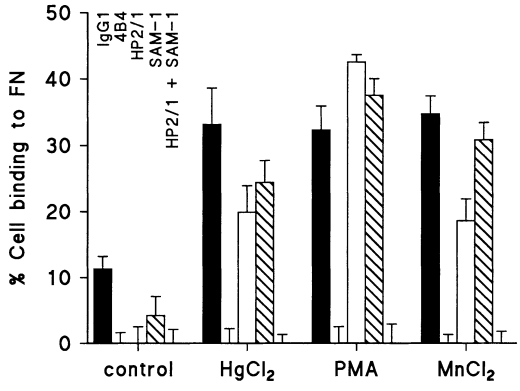

Figure 4. $HgCl_2$-induced FN adhesion of Jurkat T cells is mediated via VLA-4 and VLA-5. Jurkat T cells were preincubated with control IgG1, 4B4 (anti-CD29), HP2/1 (anti-CD49d), SAM-1 (anti-CD49e), or HP2/1 plus SAM-1. Subsequently, FN adhesion was assessed in the presence or absence of $HgCl_2$ (2·5 μM), PMA (50 ng/ml), or $MnCl_2$ (500 μM), as indicated. In separate experiments, optimal blocking concentrations of the mAb were determined, in which SAM-1 was titrated in the presence of HP2/1, and vice versa. Data represent three similar experiments.

ICAM-1, ICAM-2 and ICAM-3, as well as cell binding via VLA-4 and VLA-5 to FN. Furthermore, adhesion-promoting effects of $HgCl_2$ could be demonstrated in T lymphoblasts derived from normal human PBMC, in a human T-cell clone and in Jurkat T cells, indicating that its effect is not a unique feature of a particular cell line.

Obviously, activation of integrin-mediated ligand binding by a metal ion is not surprising.[19–21] However, comparison of the induction of Jurkat T-cell adhesion by Hg^{2+} with the effect of Mn^{2+} revealed important differences: the effect of $HgCl_2$, but not the effect of $MnCl_2$, was dependent on the presence of extracellular Ca^{2+} or Mg^{2+}, and on a physiological temperature. $MnCl_2$ supports binding of ligands to a variety of integrins. High-affinity binding sites for Mn^{2+} have been mapped in the extracellular domain of several integrins,[21,40–42] and on some of these sites, Ca^{2+} and Mg^{2+} competed for Mn^{2+} binding.[21,41,42] Mn^{2+} is able to induce ligand binding to isolated integrin molecules or integrin domains.[21,40,43] This metal ion stabilizes a high-affinity state of the integrin that resembles the ligand-bound conformation,[18] as detected by mAb which bind to specific epitopes in this conformation.[19,44] In contrast to adhesion induced by Mn^{2+}, induction of integrin-mediated ligand binding by PMA requires active cell metabolism and the presence of Ca^{2+} or Mg^{2+}, and does in general not induce a high-affinity state.[18,20,22,25] Since the requirements for induction of lymphocyte adhesion by $HgCl_2$ resemble those of PMA but not those of $MnCl_2$, we propose that $HgCl_2$ acts via an intracellular signalling pathway. Observations from our own group and from others have clearly shown that $HgCl_2$ is able to induce cell signalling events in T lymphocytes. Exposure to $HgCl_2$ can change the redox balance towards an oxidative state by decreasing the intracellular glutathione level, which can lead to signalling events such as intracellular calcium mobilization and production of oxygen radicals.[8,45] Furthermore, $HgCl_2$ can induce tyrosine phosphorylation in T lymphocytes and activation of the tyrosine kinases $p56^{lck}$ and $p60^{c\text{-}src}$.[46,47] Hg^{2+} has an extreme

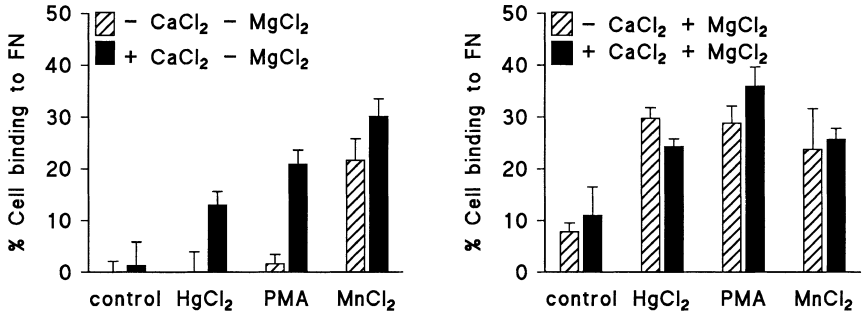

Figure 5. $HgCl_2$-induced adhesion of Jurkat T cells to FN is dependent on the presence of extracellular Ca^{2+} or Mg^{2+}. The experiment was performed as indicated in the Materials and Methods; $CaCl_2$ or $MgCl_2$ were present or absent as indicated, at a concentration of 1 mM. Data shown in (a) and (b) were obtained in a parallel experiment; they are representative for at least three experiments. Data obtained in the absence of $CaCl_2$ were confirmed by experiments in which the cells were preincubated with 1 mM EGTA, followed by extensive washing, which procedure yielded similar results (not shown).

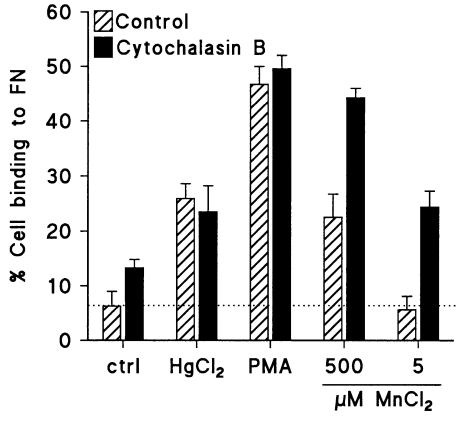

Figure 6. Modification of FN adhesion of Jurkat T cells by cytochalasin B. Cells were preincubated with cytochalasin B (5 μg/ml), followed by stimulation of the cells as indicated. Data represent two similar experiments.

high affinity for protein thiol groups, and can induce crosslinking of cystein residues on proteins.[48] Crosslinking of T-cell membrane proteins by $HgCl_2$ was proposed to trigger tyrosine phosphorylation;[46] however, these studies were performed using 10- to 1000-fold higher concentrations of $HgCl_2$ than required to support T-cell adhesion. We tested the involvement of tyrosine kinases in $HgCl_2$-induced T-cell adhesion by using the tyrosine kinase-specific inhibitor herbimycin A. This compound inhibited the induction of cell adhesion by all three stimuli used in our study (not shown) and therefore did not provide further clues about the mechanistic difference in cell adhesion induced by the stimuli examined.

Integrin activation induced by inside-out signalling has been frequently shown to require an intact cytoskeleton.[20,22] However, depending on the cell type, disruption of the actin cytoskeleton by cytochalasins can enhance LFA-1-mediated binding to ICAM-1,[27,49] probably by induction of integrin clustering on the membrane.[49,50] Kucik *et al*. have shown that exposure to PMA strongly increases the lateral movement of LFA-1 in the cell membrane, which may very well play a major role in increasing the cellular capacity to bind ligand.[27] We demonstrate here that cytochalasin B increases integrin β_1-mediated adhesion of Jurkat T cells, thus indicating that cytoskeletal anchoring regulates integrin β_1-mediated adhesion in this cell line. Furthermore, the effect of this compound was synergistically increased by $MnCl_2$, but not by $HgCl_2$ or PMA. This finding supports the hypothesis that $MnCl_2$ increases the affinity of integrins for their ligands, thereby synergizing with the action of cytochalasin B, whereas PMA and $HgCl_2$ mainly act by modification of integrin association with cytoplasmic proteins, resulting in a similar functional effect as that induced by cytochalasin B.[27]

A recent publication describes that $HgCl_2$ can induce expression of an activation-dependent epitope of integrin β_1, recognized by the mAb HUTS21, on rat lymphocytes.[51] Expression of the HUTS21 epitope is correlated to integrin adhesive function, and can be induced by various stimuli, including $MnCl_2$ and a phorbol ester.[52] These results are in line with the functional effects of $HgCl_2$ on integrin-mediated adhesion described in the present study. HUTS21 expression was also demonstrated in BN rats exposed to $HgCl_2$,[51] but the kinetics of the response suggest that lymphocyte activation secondary to treatment with $HgCl_2$ is at least partially responsible for the effects observed. A functional relevance of expression of activated integrins in $HgCl_2$-induced autoimmunity is given by an *in vivo* experiment in which a treatment with the HUTS21 mAb prevented renal inflammation and reduced autoantibody production induced by $HgCl_2$.[51]

Our study provides clear evidence that $HgCl_2$ is able to activate T lymphocytes in the absence of antigen presentation. Whether integrin-mediated ligand binding directly induced by $HgCl_2$ plays a role in its immune dysregulatory abilities *in vivo* will obviously depend on the concentration of $HgCl_2$ in the microenvironment of lymphocytes. The peak concentrations of mercury in the spleens of mice exposed to $HgCl_2$ at a dose which induces autoimmunity were reported to vary between 646 and 955 ng per gram wet tissue,[53,54] which roughly corresponds to 3·2 and 4·8 μM $HgCl_2$. These data suggest that integrin activation by $HgCl_2$ can take place *in vivo*, in the induction phase of the disease.

Integrin-mediated ligand binding is of great importance for many aspects of the immune system, including costimulation of T lymphocytes during antigen presentation, B-cell activation and antibody production, extravasation and migration of leucocytes, cell-mediated cytotoxicity and regulation of cell survival. Interactions mediated via integrin α_4 and β_1 are

required for the full development of $HgCl_2$-induced disease *in vivo*.[11,51] Therefore, we postulate that the mechanism described in the present study plays an important role in the induction and effector phases of the $HgCl_2$-induced autoimmune syndrome.

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