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Synthetic Colloids Attenuate Leukocyte–Endothelial Interactions by Inhibition of Integrin Function

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Background: **It has been suspected that synthetic colloids may interfere with leukocyte adhesion by down-regulation of endothelial cell adhesion molecules. Although inhibition of endothelial inflammation might reduce leukocyte-related tissue injury, the same mechanism may be detrimental for host defense during severe infection. Regarding the widespread use of colloids, the authors performed a laboratory investigation to determine the mechanisms by which synthetic colloids interfere with leukocyte–endothelial interactions.**

Methods: **Adhesion molecule expression on native and cytokine-activated endothelium from umbilical veins was measured after pretreatment with gelatin and various preparations of dextran or hydroxyethyl starch. Inhibition of neutrophil adhesion to activated endothelium was examined in a flow chamber by perfusion of untreated and colloid-treated neutrophils over colloid-pretreated endothelium at 2 dyn/cm^2. Comparisons were made between untreated controls, colloid-pretreated endothelium, and colloid-cotreated neutrophils.**

Results: **Intercellular adhesion molecule 1, vascular cell adhesion molecule 1, E-selectin, and P-selectin were not attenuated by any colloid. Accordingly, colloid pretreatment of endothelium alone did not reduce neutrophil adhesion. In contrast, when neutrophils were cotreated by addition of colloids to the perfusate immediately before perfusion, adhesion decreased by 31–51% ($P < 0.05$) regardless of the colloid type. As indicated by the twofold increased rolling fractions, this reduction was due to an inhibition of neutrophil integrins.**

Conclusions: **This study shows that synthetic colloids inhibit neutrophil adhesion by a neutrophil-dependent mechanism rather than interfering with endothelial cell activation. This suggests that inhibition of leukocyte sequestration by volume support is a common and transient phenomenon depending on the colloid concentration in plasma.**

VOLUME replacement with crystalloids and colloids represents a cornerstone in maintaining tissue perfusion during major surgery, trauma, shock, and sepsis. Apart from their hemodynamic properties, it has been repeatedly suggested that synthetic colloids interfere with endothelial dysfunction,[1–7] but the results of various studies are inconsistent. Although hydroxyethyl starches (HESs) attenuated capillary leakage, soluble adhesion molecules, leukocyte adhesion, and cytokine concentrations *in vivo* and *in vitro*,[1–3,7–9] HES was also reported to be less potent than dextrans or even without beneficial effects on leukocyte-related reperfusion injury in other studies.[5] Gelatin has not been as extensively studied, and results are conflicting regarding its microcirculatory effects.[9,10] Studies on leukocyte function are also inconsistent, showing a severalfold increase in the leukocyte integrin-epitope CD18 after hemodilution of whole blood with dextran and HES,[11] whereas no changes in leukocyte adhesion molecules were reported in another comparative study examining lactated Ringer's solution, albumin, gelatin, and HES.[12]

Expression of functionally intact adhesion molecules is essential for leukocyte–endothelial interactions under flow.[13–23] In cytokine-activated endothelium, E-selectin is synthesized by *de novo* protein synthesis and is expressed on the endothelial cell membrane a few hours later.[14,15] P-selectin is constitutively present in intracellular granules of platelets and endothelial cells. It is translocated to the cell surface within minutes after trauma or activation with agonists such as thrombin.[15,24] L-selectin, the third member of the selectin family, is constitutively expressed on circulating leukocytes and is shed from the cell surface early after activation or binding to the endothelium.[25] Binding of selectins to carbohydrate structures on glycoprotein ligands on leukocytes leads to the initial tethering of circulating leukocytes. These rolling interactions are mandatory for initial leukocyte–endothelial interactions under shear and subsequent activation of leukocyte integrins.[14,15,25,26] CD11a/CD18 (lymphocyte function-associated antigen 1 [LFA-1]) and CD11b/CD18 (Mac-1) are the most important members of the β_2 integrin family. Their function is largely controlled by qualitative changes in affinity and avidity after activation, whereas quantitative changes in expression play a minor role.[27–30] Upon activation, both integrins bind to endothelial adhesion molecules of the immunoglobulin gene superfamily, such as intercellular adhesion molecule 1 (ICAM-1), thus leading to firm adhesion.[14,17,27–30] Apart from their physiologic role in immune defense, adhering leukocytes largely contribute to leukocyte-related vascular injury. Therefore, inhibition of adhesion molecules has been advocated to reduce organ dysfunction during shock and ischemia–

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reperfusion.[31–34] At the same time, however, deficiency or inhibition of endothelial selectins, ICAM-1, or leukocyte β_2 integrins may result in immunosuppression and worsened outcome during severe infection.[13,15,35,36] Because traumatized patients are at risk for both,[37] it is striking that the mechanisms by which synthetic colloids may interfere with leukocyte-endothelial interactions are largely unknown. Most studies to date focused only on subsets of adhesion molecules,[38] examined surrogate parameters such as von Willebrand factor (vWF),[38] used simplified static adhesion assays, or did not account for functional changes in leukocyte receptor activity.[4,7,11,12]

Leukocyte adhesion is largely influenced by overlapping functions of selectin adhesion molecules, intravascular shear forces, and activation of β_2 integrins.[14,15–23,27–30] Therefore, these studies provide no conclusive evidence for the common hypothesis that certain synthetic colloids may attenuate leukocyte sequestration by a direct interaction of specific polysaccharide structures with the endothelium.[1–5,7] Regarding the large variety of pharmacologic modifications in clinically available volume expanders and the important role of leukocyte adhesion in organ injury and immunity,[13,14,31–36] direct interferences of certain colloid structures with leukocyte–endothelial interactions may influence the timing and type of volume support chosen. Therefore, we studied the effects of the most commonly used synthetic colloids on cytokine-activated endothelial cell adhesion molecules and leukocyte adhesion. We examined leukocyte–endothelial interactions in a dynamic adhesion assay, allowing us to discriminate between effects on adhesion molecule expression and molecule function under physiologic postcapillary flow conditions.

Materials and Methods

Endothelial Cell Culture and Leukocyte Separation

Human cells were derived from human umbilical veins or from blood donations of healthy volunteers after obtaining institutional and ethical committee approval (Ethical Committee of the Faulty of Medicine, University of Tuebingen, Tuebingen, Gemany) and informed consent as previously described.[21,22,39,40] Human umbilical venous endothelial cells (HUVECs) were harvested from umbilical cords by collagenase treatment (0.1% collagenase A; Boehringer, Mannheim, Germany) and cultured in endothelial cell growth medium (PromoCell, Heidelberg, Germany) on collagen-coated cell culture dishes (Falcon Biocoat; Becton Dickinson Labware, Bedford, MA) or rectangular coverslips (Kindler, Freiburg, Germany) coated with rat tail collagen I (Falcon Biocoat). Confluent HUVECs of the first and second passage were used for the experiments. Polymorphonuclear neutrophils (PMNs) were isolated from freshly drawn citrated blood by density gradient centrifugation at 1,700 rpm on a discontinuous Percoll gradient with 63% and 72% Percoll in phosphate buffered saline (1.130 g/ml Percoll; Amersham Pharmacia Biotech, Uppsala, Sweden). The bottom layer, containing the PMNs, was collected, and contaminating erythrocytes were removed by hypotonic lysis in 10% NH_4Cl on ice. After a final wash with phosphate-buffered saline (Sigma, St. Louis, MO), the PMN pellet was resuspended in cold Medium 199 (Sigma) at 5×10^7/ml until immediately before the flow experiment. As determined by trypan blue exclusion, staining for vWF and cell surface antigen analysis both separation protocols yielded functionally intact, nonactivated HUVECs and PMNs at greater than 90% purity.[22,39,40]

Experimental Protocol

To address the effects of various colloids on endothelial cell adhesion molecule expression, HUVECs were incubated with 5 mg/ml of HES 450 (molecular weight in kilodaltons), HES 200, HES 130, HES 70, dextran 70, dextran 40, or gelatin dissolved in medium. This concentration resembles plasma concentrations during the first 6 h after bolus infusion of 500–1,000 ml of the tested volume expanders.[41–43] HES 450 (degree of substitution 0.7), HES 200 (degree of substitution 0.5), HES 130 (degree of substitution 0.4), and dextran 70 were purchased from Fresenius Kabi (Bad Homburg, Germany). HES 70 (degree of substitution 0.5) was obtained from Baxter (Erlangen, Germany), dextran 40 was obtained from Delta-Pharma (Pfullingen, Germany), and urea-linked gelatin was obtained from Aventis Pharma (Bad Soden, Germany). Two hours after exposure to colloids, HUVECs were activated with tumor necrosis factor α (TNF-α; Sigma) at 0.5 ng/ml for 4 h or were left nonactivated so that a total colloid incubation of 6 h was achieved in all experiments (table 1). Because activation with TNF-α does not result in sustained up-regulation of P-selectin on human endothelium,[44] we examined its expression on thrombin-activated HUVECs. Therefore, in separate experiments, HUVECs were activated with 2 units/ml thrombin (Sigma) during the last 2 min of the 6-h colloid incubation. Within each activation, the colloid-pretreated HUVECs were compared with untreated controls without colloids (table 1). At the end of each incubation period, the cells were washed and stained with saturating amounts of fluorochrome-conjugated monoclonal antibodies against vascular cell adhesion molecule 1 (VCAM-1), E-selectin (both fluorescein conjugates; Southern Biotechnologies Associates, Birmingham, AL), ICAM-1 (phycoerythrin-conjugate; Immunotech, Marseille, France), and P-selectin (primary antibody immunoglobulin [Ig] G1, mouse anti-human and secondary antibody Fab_2 IgG, goat anti-mouse, phycoerythrin-conjugate; Immunotech). After antibody staining, the HUVECs were trypsinized and immediately analyzed for adhesion molecule expression in a flow cytometer (FACSort; Becton Dickinson, San Jose, CA).

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Table 1. Experimental Groups

Experimental Group	Cell Activation	Colloid Treatment	Analysis
Control (untreated HUVECs)	Nonactivated Activated	None	Flow cytometry
Colloid-pretreated HUVECs	Nonactivated Activated	6 h, 5 mg/ml	Flow cytometry
Control (untreated)	Nonactivated Activated (HUVECs)	None	Adhesion assay
Colloid-pretreated HUVECs with untreated PMNs	Activated (HUVECs)	6 h, 10 mg/ml (HUVECs) None (PMNs)	Adhesion assay
Colloid-pretreated HUVECs with cotreated PMNs	Activated (HUVECs)	6 h, 10 mg/ml (HUVECs) 10 min, 10 mg/ml (PMNs)	Adhesion assay

Colloid treatment and cell activation of the different experimental groups. Expression of E-selectin, P-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) was analyzed by flow cytometry on nonactivated human umbilical venous endothelial cells (HUVECs) and on activated HUVECs after activation with either tumor necrosis factor α (TNF-α, 4 h at 0.5 ng/ml; E-selectin, ICAM-1, VCAM-1) or thrombin (2 min at 2 units/ml; P-selectin) as detailed in the Materials and Methods. Effects of colloids were examined by comparisons between controls and colloid-pretreated HUVECs. In functional adhesion assays, TNF-α–activated HUVECs (4 h at 0.5 ng/ml) were perfused with nonactivated polymorphonuclear neutrophils (PMNs). Comparisons were made between activated, untreated controls; activated, colloid-pretreated HUVECs with untreated PMNs; and colloid-pretreated HUVECs with cotreated PMNs. Nonactivated cocultures were used only to assess the effect of TNF-α on PMN adhesion.

Functional Adhesion Assay

To determine the mechanisms by which certain synthetic colloids may inhibit leukocyte–endothelial interactions, we examined the effects of HES 200, HES 130, dextran 70, dextran 40, and gelatin on PMN adhesion under a postcapillary shear stress of 2 dyn/cm^2. Adhesion of PMNs to untreated, cytokine-activated HUVECs and colloid-pretreated, cytokine-activated HUVECs were compared. To examine immediate effects on bond formation between the interacting cells, a third experimental group consisting of colloid-pretreated HUVECs and cotreated PMNs was introduced. In this group, the PMNs were resuspended in a colloid-supplemented medium immediately before the adhesion assay (table 1). To guarantee constant PMN concentrations (10^6 cells/ml in Medium 199) in all experiments, dry substances of the colloids were dissolved in medium instead of using colloid solutions. Cytokine activation and colloid pretreatment were identical to the protocol described above, with one modification. Because we also wanted to address early effects of colloids on bond formation between leukocytes and the endothelium rather than endothelial pretreatment alone, we increased the colloid concentration to 10 mg/ml in all functional assays to account for the higher initial plasma concentrations of volume expanders during infusion.[41–43] PMN adhesion was quantified in a parallel plate flow chamber as previously reported.[21,22,39,40] In brief, HUVEC-covered coverslips were placed into a heatable flow chamber, rinsed with Medium 199, and perfused at 37°C with the PMN suspension for 10 min at a shear stress of 2 dyn/cm^2. The flow rate Q in milliliters per second necessary to produce the desired shear stress τ_w could be calculated according to the formula $Q = (h^2\, w\tau_w)/6\mu$, in which h is the chamber height (0.015 cm), w is the width (1.25 cm), and μ is the viscosity of the perfusate (0.007 poise for isolated PMNs in Medium 199, 0.007–0.009 poise for colloid-supplemented medium) as measured in a cone and plate viscometer (DV-III+; Brookfield Engineering Laboratories, Middleboro, MA). Using phase-contrast microscopy (20× objective, DMIRB; Leica, Bensheim, Germany), PMN adhesion was determined from digitized 10-s video recordings of five different fields of view at the end of each 10-min perfusion period. According to Lawrence *et al.*,[19,23] PMNs were defined as rolling when traveling below 40 μm/s. A PMN moving less than one cell diameter in 10 s was defined to be firmly adherent. As a measure for adhesion efficiency,[20,27] the rolling fraction was calculated as [(No. of rolling cells) × 100]/(No. of rolling cells + No. of firmly adherent cells). Using this equation, it could be distinguished whether inhibition of PMN adhesion occurred by inhibition of selectin-mediated rolling interactions or inhibition of integrin-mediated firm adhesion to endothelial ICAM-1.[27,28] The mean rolling velocities were determined from more than 25 individual PMN-velocity profiles for each experimental condition as derived from a customized software for image recognition (CellTracker; Christof Zanke, University of Tuebingen, Tuebingen, Germany).

Statistics

The medians of fluorescence intensity (MFIs) were calculated from 5,000 single events detected by the flow cytometer for each sample of five experiments. For statistical analysis, an analysis of covariance (ANCOVA) was performed to determine whether adhesion molecule expression was influenced by the cell culture (modeled as random effect), TNF-α activation, colloid pretreatment (continuous factors), or whether an interaction between TNF-α–activation and colloid pretreatment occurred. To analyze effects of individual colloids, ratios between colloid-pretreated samples and untreated controls (colloid/control) were calculated for each of the various colloid preparations. Significant colloid effects were assumed for those ratios whose 95% confidence intervals did not include 1. Firm adhesion and rolling fractions were ana-

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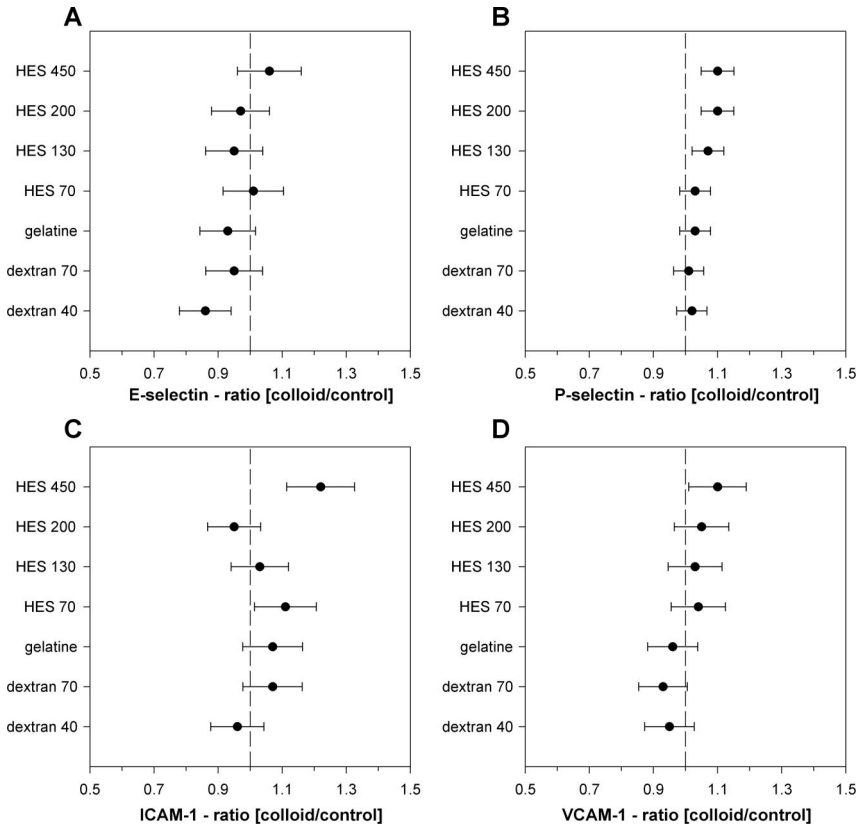

Fig. 1. Ratio of adhesion molecule expression on nonactivated, colloid-pretreated endothelium (colloid) related to nonactivated, untreated endothelium (control). Colloid-pretreated endothelial cells were incubated for 6 h with the indicated colloids at 5 mg/ml. Controls were kept in medium alone. Expression of E-selectin (*A*), P-selectin (*B*), intercellular adhesion molecule 1 (ICAM-1; *C*), and vascular cell adhesion molecule 1 (VCAM-1; *D*) determined from flow cytometric analysis of 5,000 events/sample. Ratios (colloid/control) are presented as medians and their 95% confidence intervals, calculated from five experiments (analysis of covariance). Statistical significance on the 5% level was assumed for those ratios whose 95% confidence intervals did not include 1 (indicated by *reference line*). Colloid preparations of different molecular weights are presented with their molecular weight in kilodaltons (*e.g.*, hydroxyethyl starch as HES 450).

lyzed from the mean of five randomly chosen fields of view of six experiments. Effects of colloid treatment (either pretreatment of HUVECs with and without cotreatment of PMNs) were compared by ANCOVA and *post hoc* paired *t* tests using the Bonferroni-Holm correction for multiple comparisons. For presentation, ratios (colloid/TNF-α–activated control) were calculated, which are presented as means and their corresponding 95% confidence intervals. All analyses were performed using the statistical software package JMP (SAS Institute Inc., Cary, NC).

Results

Adhesion Molecule Expression on Nonactivated and Activated HUVECs

Nonactivated HUVECs expressed small constitutive amounts of ICAM-1 but did not show expression of endothelial selectins or VCAM-1. After activation with TNF-α, a 10-fold up-regulation of E-selectin, ICAM-1, and VCAM-1 was observed. P-selectin increased by 60% after activation with thrombin (ANCOVA, $P < 0.05$; not shown).

Effects of Colloids on Adhesion Molecule Expression

On nonactivated HUVECs, small increases in P-selectin, ICAM-1, and VCAM-1 were observed for some starch preparations (ANCOVA, $P < 0.05$). HES 450 increased all three adhesion molecules by 10–20%. HES 200 and HES 130 increased P-selectin by 10%, whereas HES 70 increased ICAM-1 by 10% (fig. 1).

None of the tested colloids reduced the expression of any adhesion molecule on activated HUVECs. In contrast, a 21% increase in E-selectin and a 15% increase in ICAM-1 were noted after treatment with gelatin. Dextran 70 increased the expression of both adhesion molecules by 11% and 15%, respectively (fig. 2; ANCOVA, $P < 0.05$).

Effects of Colloids on PMN Adhesion

Activation of HUVECs with TNF-α largely increased PMN adhesion under a postcapillary shear stress of 2 dyn/cm^2 by 10- to 20-fold (460 $\pm$ 35 PMNs/mm^2) when compared with nonactivated HUVECs (not shown). Pretreatment of HUVECs with HES 200, HES 130, gelatin, dextran 70, and dextran 40 had no significant effect on firm adhesion, rolling fraction, or rolling velocity (fig. 3 and tables 2 and 3). In contrast, cotreatment of PMNs immediately before perfusion showed significant reductions in firm adhesion for all colloids tested (fig. 3; ANCOVA, $P < 0.05$). Dextran 40 and dextran 70 reduced adhesion by 31% and 51%, respectively. Treatment with gelatin resulted in a 46% decrease. HES 200 and HES 130 attenuated firm adhesion by 43–50%. Together with the decrease in firm adhesion, twofold increased rolling fractions were observed (table 2; ANCOVA, $P < 0.05$), indicating that rolling interactions were maintained whereas the transition to firm adhesion

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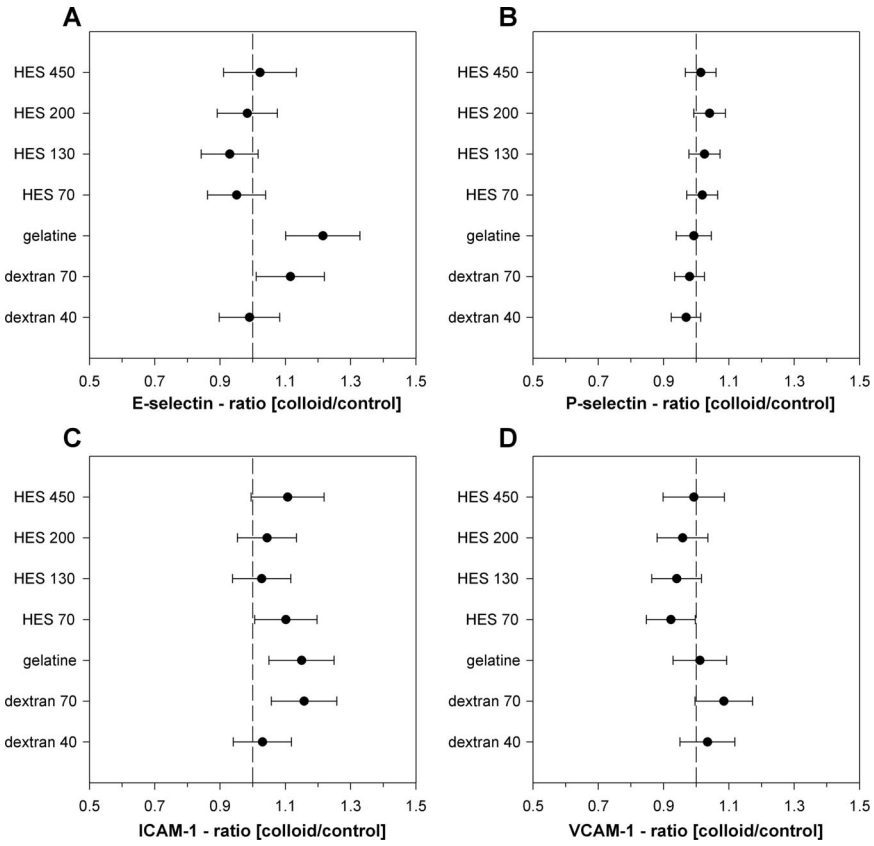

Fig. 2. Ratio of adhesion molecule expression on colloid-pretreated endothelium (colloid) related to untreated endothelium (control) after activation with tumor necrosis factor α (*A*, *C*, and *D*) or thrombin (*B*). Starting 2 h before addition of tumor necrosis factor α (0.5 ng/ml), colloid-pretreated endothelial cells were incubated with the indicated colloids at 5 mg/ml throughout the complete period of cytokine activation (4 h). On thrombin-activated endothelium, thrombin was added at 2 units/ml for the last 2 min of the 6-h colloid incubation. Untreated controls were activated with tumor necrosis factor α or thrombin in medium accordingly. Expression of E-selectin (*A*), P-selectin (*B*), intercellular adhesion molecule 1 (ICAM-1; *C*), and vascular cell adhesion molecule 1 (VCAM-1; *D*) determined from flow cytometric analysis of 5,000 events/sample. Ratios (colloid/control) are presented as medians and their 95% confidence intervals, calculated from five experiments (analysis of covariance). Statistical significance on the 5% level was assumed for those ratios whose 95% confidence intervals did not include 1 (indicated by *reference line*). Colloid preparations of different molecular weights are presented with their molecular weight in kilodaltons (*e.g.*, hydroxyethyl starch as HES 450).

became impaired as soon as the colloids were added to the PMN perfusate. Accordingly, the rolling velocities were slow and showed no relevant differences between the treatment groups (table 3). As determined by viscometry, the changes in viscosity were small after addition of the colloids (0.007–0.009 poise) and did not correlate with the observed reduction in firm adhesion.

Discussion

The recruitment of leukocytes to areas of infection or tissue damage is one of the most important functions of the innate immune system.[14] Because postcapillary wall shear stress opposes cell interactions on quiescent endothelium, up-regulation of endothelial cell adhesion molecules and binding to their leukocyte counterreceptors are prerequisites for the emigration of leukocytes into areas of inflammation.[13–17] When considering the slow turnover rate of endothelial cells and the prolonged intracellular storage of synthetic colloids such as HES,[40,45] it is, therefore, most important that none of the tested colloids attenuated leukocyte adhesion by direct down-regulation of endothelial cell adhesion molecules. Furthermore, because PMN adhesion to colloid-pretreated HUVECs remained unchanged, it seems that colloid treatment of the endothelium did not interfere with endothelial chemokines relevant for adhesion. The unchanged expression of E-selectin and ICAM-1 on HUVECs pretreated with HES 200 and HES 130 is consistent with previous studies on diafiltered medium-molecular-weight starch fractions or HES 200.[38,40] However, direct effects of other synthetic colloids on endothelial cell adhesion molecule expression have not yet been investigated. In addition, the direct effects of synthetic colloids on VCAM-1 have not been studied, except for measurements of soluble adhesion molecules in patients.[8] Expression of P-selectin, in contrast, has been suspected to become attenuated by HES 450.[38] However, this assumption is based on the finding that HES 450 decreased the release of vWF in HUVECs after activation with thrombin. Because vWF and P-selectin are stored in the same intracellular granules, it was hypothesized that the decreased release of vWF after pretreatment with HES 450 may indicate a concomitant decrease in P-selectin.[38] We measured P-selectin directly, and our results clearly argue against this hypothesis. Because selectins bind to carbohydrate structures, it has been further suspected that specific polysaccharides such as HES interfere with selectin binding by mechanisms involving molecular weight, substitution with hydroxyethyl groups, or both.[1,4] However, the well-preserved rolling interactions in our experiment show that this is not the case. On colloid-pretreated HUVECs, no interferences with PMN adhesion were shown for any colloid tested. In addition, the functional adhesion assay revealed that the minor increase in certain adhesion

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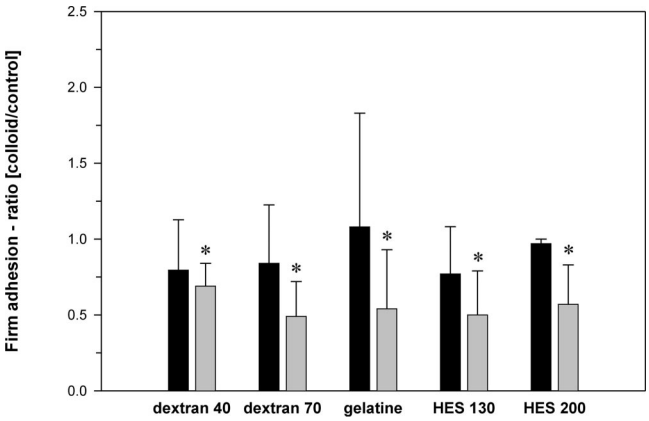

Fig. 3. Effect of different colloid-treatments on neutrophil (PMN) adhesion to cytokine-activated endothelium. Firm adhesion is presented as ratios (colloid/control) and upper 95% confidence intervals, calculated from means of six experiments (absolute mean of cytokine-activated control, 460 PMNs/mm^2; 95% confidence interval, ±35). Starting 2 h before addition of tumor necrosis factor α (0.5 ng/ml), colloid-pretreated endothelial cells were incubated with the indicated colloids at 10 mg/ml throughout the complete period of cytokine activation (4 h). In the group containing colloid-pretreated endothelium and untreated PMNs (*black columns*), only the endothelial cell culture medium was supplemented with the indicated colloids. In the group containing colloid-pretreated endothelium and cotreated PMNs (*gray columns*), the colloids were also added to the PMN suspension. The cytokine-activated controls, necessary to calculate the ratios (colloid/control), were activated with tumor necrosis factor α in medium alone. Firm adhesion was quantified from means of five different fields of view for each experimental condition as detailed in the Materials and Methods. Effects of colloid treatment were analyzed by an analysis of covariance (* $P < 0.05$ *vs.* control, *post hoc t* test). Colloid preparations of different molecular weights are presented with their molecular weight in kilodaltons (*e.g.*, hydroxyethyl starch as HES 200).

molecules after pretreatment with dextran 70 or gelatin did not translate to an increased adhesion.

The finding that gelatin increased E-selectin and ICAM-1 on activated HUVECs by 21% and 15% without increasing PMN adhesion is in accord with previous results on cell interactions at defined E-selectin site densities. In that investigation, the rolling of transfected cells was not changed to a relevant extent unless E-selectin site density was more than doubled.[18] In our study, the relatively large upper confidence interval (1.8-fold adhesion) found on gelatin-pretreated HUVECs may reflect some greater degree of endothelial activation when compared with the other colloids. However, this finding was not statistically significant, and its clinical relevance is clearly offset by the 46% decreased adhesion after cotreatment of the PMNs. On nonactivated HUVECs, gelatin slightly increased the basal expression of ICAM-1 but did not induce any up-regulation of inducible E-selectin. This suggests that gelatin is a relatively weak stimulus for endothelial cells that does not result in substantial expression of adhesion molecules unless the endothelium is activated by other agonists. The mechanism by which gelatin might have contributed to TNF-α–induced endothelial activation remains unknown. Although gelatin

Table 2. Rolling Fractions of PMNs on TNF-α–activated HUVECs at 2 dyn/cm^2

	Rolling Fraction, % of Total Adherent PMNs	
	Pretreated HUVECs	Cotreated PMNs
TNF-α control	6.6 ± 1.2	NA
Dextran 40	10.7 ± 2.0	18.2 ± 1.4
Dextran 70	6.0 ± 0.9	17.9 ± 5.0
Gelatin	6.3 ± 1.3	11.8 ± 2.4
HES 130	10.7 ± 3.0	13.3 ± 2.8
HES 200	5.6 ± 1.2	13.0 ± 1.6

Data are presented as mean ± 95% confidence interval from six experiments. Rolling fractions of polymorphonuclear neutrophils (PMNs) on tumor necrosis factor α (TNF-α)–activated controls of human umbilical venous endothelial cells (HUVECs), colloid-pretreated HUVECs (10 mg/ml), and colloid-pretreated HUVECs with cotreated PMNs (10 mg/ml) were calculated as described in the Materials and Methods. As determined by an analysis of covariance, cotreatment of PMNs significantly increased rolling fractions compared with TNF-α controls or pretreatment of HUVECs alone ($P < 0.01$). Colloid preparations of different molecular weights are presented with their molecular weight (*e.g.*, hydroxyethyl starch as HES 200).

NA = not applicable.

contains proteins of the extracellular matrix, such as collagen, which have been shown to induce signal transduction pathways in different cell populations,[46,47] we are not aware of any study that has addressed the effects of gelatin on endothelial signaling.

In contrast to the negligible effects of synthetic colloids on endothelial cell adhesion molecule expression, cotreatment of PMNs with colloids before perfusion immediately reduced adhesion regardless of the colloid type. The finding that biochemically different polysaccharides and polypeptides caused similar inhibition suggests that these synthetic macromolecules act by means of a direct, unspecific inhibition of PMN binding rather than by interfering with endothelial cell activation. Because firm adhesion is largely controlled by functional changes in integrin avidity and affinity rather than quan-

Table 3. Rolling Velocities of PMNs on TNF-α–activated HUVECs at 2 dyn/cm^2

	Rolling Velocity, μm/s	
	Pretreated HUVECs	Cotreated PMNs
TNF-α control	1.5 ± 0.6	NA
Dextran 40	1.5 ± 0.6	1.7 ± 1.1
Dextran 70	1.9 ± 0.7	1.1 ± 0.3
Gelatin	1.4 ± 0.3	2.7 ± 1.9
HES 130	1.5 ± 0.6	2.7 ± 2.8
HES 200	1.0 ± 0.3	1.3 ± 0.3

Data are presented as mean ± 95% confidence interval calculated from more than 25 individual velocity profiles of rolling polymorphonuclear neutrophils (PMNs) on tumor necrosis factor α (TNF-α)–activated controls of human umbilical venous endothelial cells (HUVECs), colloid-pretreated HUVECs (10 mg/ml), and colloid-pretreated HUVECs with cotreated PMNs (10 mg/ml) as described in the Materials and Methods. No statistical differences were observed between the groups (analysis of covariance). Colloid preparations of different molecular weights are presented with their molecular weight (*e.g.*, hydroxyethyl starch as HES 200).

NA = not applicable.

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titative cell surface expression,[27–30] we abstained from measuring integrin expression by flow cytometry. To measure integrin function, we calculated rolling fraction instead, which is an established parameter for adhesion efficiency and integrin activation.[20,27,28] The twofold increased rolling fractions show that the presence of dextrans, HESs, and gelatin in the perfusate immediately impaired the integrin-dependent transition of rolling into firm adhesion, whereas selectin-mediated rolling interactions were not affected.

These findings are in accord with a previous report on static adhesion of T cells to mouse endothelioma cells.[48] Examining dextran 40, the authors observed a dose-dependent reduction in T-cell adhesion only when the lymphocytes were pretreated with dextran 40 but not after pretreatment of the endothelium. Using flow cytometry and confocal microscopy, they showed that dextran 40 inhibits the adhesion-induced clustering of LFA-1, thereby attenuating the activation-dependent avidity of this integrin without any change in quantitative surface expression. For HES, however, conflicting findings have been reported. Consistent with our recent results, endothelial pretreatment alone with HES 200 for up to 48 h did not attenuate PMN adhesion to cytokine-activated HUVECs under flow.[40] In contrast, others reported a 24% reduced PMN-transmigration across HES-pretreated HUVECs during 3 h of activation with a chemotactic stimulus in a static adhesion assay.[7] However, unlike dynamic adhesion assays, static assays lack selectin function which operate only under flow conditions.[18] Because integrin activation is a short-lived process peaking during preceding selectin interactions,[26,30] static assays and prolonged activation with chemotactic agents do not necessarily mirror leukocyte–endothelial interaction *in vivo*. Therefore, our findings obtained at postcapillary shear stress and the observation of an altered integrin clustering after dextran 40[48] strongly suggest that all types of synthetic colloids impair leukocyte adhesion by a common inhibition of integrin function.

In contrast to many studies *in vivo*, our results are derived from comparisons to medium and not to crystalloid or albumin controls. Unlike *in vivo*, however, there was no need for a control of hemodilutional effects in our *in vitro* assay. Because purified dry substances of the different colloids were used, the PMN concentrations remained constant before and after adding the colloids. *In vivo*, PMN adhesion is mainly determined by molecular interactions of adhesion molecules but also seems to be modulated by unspecific mechanisms like erythrocyte aggregation.[49] Although erythrocyte rheology might be altered differently by crystalloids and colloids,[50] these interferences did not play a role for the adhesion of isolated PMNs in our assay. Most importantly, the addition of colloids did not affect the concentrations of nutrients and cations as confirmed by measuring sodium, potassium, calcium, and magnesium in colloid supplemented medium. Dilution of the medium with crystalloids, in contrast, alters the concentrations of these molecules. Regarding the importance of divalent cations for selectin- and integrin-mediated interactions,[28,51] comparisons to crystalloids *in vitro*, therefore, result in confounding effects rather than representing a valid control for interactions of adhesion molecules. For similar reasons, we abstained from using an albumin group. Albumin exerts its controversially discussed antiinflammatory effects *in vivo* through a variety of mechanisms, such as binding to the interstitial matrix, interferences with erythrocyte deformability and shear stress, or through transport of fatty acids, nitric oxide, and metals, with resultant scavenging of reactive oxygen species.[52–56] Regarding leukocyte sequestration into sites of infection, these mechanisms are rather unspecific when compared with adhesion molecule function. Adhesion molecule expression, in contrast, might be decreased or even increased by human albumin.[8,38,57,58] In accord with the well-documented proinflammatory effects of certain albumin batches,[59,60] we have shown previously that different albumin preparations activate E-selectin, ICAM-1, and VCAM-1 differently depending on their manufacture.[58] These observations show that the effects of albumin preparations are not the same among each other and largely question the relevance of human albumin as a valid control for molecular interactions in adhesion assays.

Although flow chamber–based, *in vitro* models cannot simulate all parameters of the human microcirculation, a number of experiments with blocking antibodies have shown that the mechanisms of PMN adhesion in the parallel plate flow chamber are largely identical to those that have been observed *in vivo*.[15–23,31,33,34,61] Therefore, our finding of a common, direct inhibition of integrin-dependent PMN adhesion to cytokine-activated endothelium *in vitro* might have several relevant implications for leukocyte sequestration *in vivo*. First, the immediate inhibition of PMN adhesion suggests that adhesion will also be immediately inhibited *in vivo* and does not require pretreatment before a microcirculatory insult. Second, the observation that all types of synthetic colloids attenuated adhesion similarly indicates that the type of volume expander does not play a major role in the acute inhibition of leukocyte–endothelial interactions. Finally, because colloids reduced adhesion by a PMN-dependent mechanism rather than interfering with the slowly regenerating endothelium, it seems that the duration of this effect will be largely determined by the intravascular elimination of the colloid. Regarding the susceptibility of traumatized patients to both leukocyte-related tissue injury and infection,[37] we conclude that the clinically relevant effect of synthetic colloids on leukocyte sequestration depends on the regulation of integrin function and thus their pharmacokinetic pro-

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files, but not on specific interactions with the endothelium.

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