To explore the molecular basis of von Willebrand factor (VWF) clearance, an experimental model employing VWF-deficient mice was developed. Biodistribution was examined by the injection of radiolabeled VWF, which was primarily directed to the liver with minor amounts in other organs. Disappearance of VWF from plasma was characterized by a rapid initial phase (t_{1/2,α} = 13 min) and a slow secondary phase (t_{1/2,β} = 3 h), with a mean residence time (MRT) of 2.8 h. A similar clearance was observed for VWF consisting of only high or low molecular weight multimers, indicating that, in our experimental model, clearance is independent of multimeric distribution. This allowed us to compare the survival of full-length VWF to truncated variants. Deletion of both the amino-terminal D3-D3 and carboxyl-terminal D4-CK domains resulted in a fragment with a similar clearance to wild-type VWF. Deletion of only the D3 region was associated with an almost 2-fold lower recovery and increased clearance (MRT = 0.6 h), whereas deletion of only the D4-CK region resulted in a significantly reduced clearance (MRT = 4.5 h, p < 0.02). These results point to a role of the D3-D3 region in preventing clearance of VWF. Furthermore, replacement of D3 domain residue Arg-1205 by His resulted in a markedly increased clearance (MRT = 0.3 h; p = 0.004). Therefore, this mutation seems to abrogate the protective effect of the D3-D3 region. In vitro analysis of this mutant also revealed a 2-fold reduced affinity for VWF propeptide at low pH, showing that mutation of Arg-1205 results not only in an increased clearance rate but is also associated with an impaired pH-dependent interaction with VWF propeptide.

von Willebrand factor (VWF) is a multimeric plasma protein that participates in the hemostatic process. The absence of functional VWF is associated with an abnormal bleeding tendency known as von Willebrand Disease (VWD) (1, 2). VWF contributes to hemostasis in a dual manner. First, it promotes the adhesion of platelets at sites of vascular injury by acting as a molecular bridge between the sub-endothelial collagen matrix and the platelet-surface receptor complex glycoprotein (Gp) Ibα/IX/V (3). In addition, VWF serves as a carrier protein for coagulation factor VIII (FVIII) in the circulation. This chaperone function results in stabilization of the FVIII heterodimeric structure (4) and protection of FVIII from premature clearance by the low density lipoprotein receptor-related protein (5, 6).

VWF is produced and stored in endothelial cells and megakaryocytes. It is synthesized as pre-pro-VWF, a single chain polypeptide with the domain structure D1-D2-D3-D4-A2-A3-D4-B1-B2-B3-C1-C2-CK (7). The pro-VWF molecule is subject to extensive posttranslational modifications, including carboxyl-terminal dimerization (8). Because of proteolytic processing, pro-VWF is further separated into two polypeptides, the VWF propeptide (also known as VWF AgII) containing the D1-D2 region, and mature VWF, which comprises the remaining domains. The main function of the propeptide is to facilitate amino-terminal multimerization of mature VWF within the post-Golgi compartments and its targeting to Weibel-Palade storage bodies in endothelial cells (9, 10). Thus, the propeptide and mature VWF appear to interact within cellular organelles, where conditions are slightly acidic (pH < 6.5). The extent of multimerization may differ between VWF molecules and is defined by the number of dimeric subunits included (between 2 and >20). As a result, VWF circulates within plasma as a heterogeneous sized protein with its molecular mass ranging from 0.5 × 10^6 to over 5 × 10^6 Da.

The average plasma level of VWF is ~10 μg/ml (35 nM based on monomer concentrations), although a broad range is observed between individuals (11). A major part of this variation is determined by ABO blood type; the average VWF levels in persons with blood group O are ~25% lower than those in non-O individuals (12). However, other genetic (13, 14) and non-genetic factors, such as age and physical activity (15, 16), may also contribute to this variation. These factors may influence VWF levels by modulating the balance between its biosynthesis and clearance. In the last two decades, biosynthesis of VWF has been well studied (for recent reviews see Refs. 17 and 18). In contrast, little is known about the mechanism by which VWF is cleared from the circulation. However, this issue is becoming increasingly important, as illustrated by several recent reports suggesting that modified clearance plays a role in the pathogenesis of VWD type 1 (19, 20).
One of the very few parameters that has been reported to date to influence VWF clearance is the VWF glycosylation profile (12, 21–24). For instance, by using an animal model it has been shown that, in the absence of the enzyme ST3Gal-IV, the half-life of endogenous VWF is reduced 2-fold (24). Moreover, in a patient group referred to the hospital for real or suspected bleeding disorder, reduced ST3Gal-IV-mediated sialylation was observed to be associated with reduced VWF plasma levels (24). These findings suggest that carbohydrate components play a role in the clearance of VWF.

In addition to the carbohydrate side chains, polypeptide regions are also likely to contribute to the interaction with clearance receptors. However, no such region has yet been identified within the VWF molecule. Thus, the molecular basis of VWF clearance is still an unexplored issue in view of the responsible receptors as well as in view of the regions within VWF that mediate binding to these receptors. To gain more insight into the molecular aspects of VWF clearance, an experimental model was developed employing mice genetically deficient for VWF. Using this model, the in vivo survival of truncated as well as mutated variants of VWF was compared with that of full-length VWF. These experiments indicate that the D3-D3 region of VWF and, in particular, the D3-domain residue Arg-1205 contribute to the clearance process of VWF.

**EXPERIMENTAL PROCEDURES**

**Mice**—The VWF-deficient mice (25) and wild-type mice used in this study were on a C57BL/6J background and were used between 8 and 12 weeks of age. Housing and experiments were done as recommended by French regulations and the experimental guidelines of the European Community.

**Proteins**—For biodistribution experiments, a highly purified, plasma-derived (pd) human VWF concentrate (a kind gift of Dr. C. Mazurier, LRP, Lille, France) was labeled with Na251I (Amersham Biosciences) using the IODO-GEN method (Pierce). Routinely, this method resulted in preparations containing 2–4 × 10^6 cpm/μg VWF. Free iodine in final preparations was <5% as determined by precipitation with 20% trichloroacetic acid. VWF preparations enriched in either high or low molecular weight multimers were obtained by fractionating an intermediate pure VWF concentrate (Hamate-P, Behring, Marburg, Germany) by gel filtration chromatography using Bio-Gel A 1.5m (Bio-Rad). The factor VIII light chain was kindly provided by Dr. K. Mertens (Sanquin Research, Amsterdam, The Netherlands). Recombinant GpIbα (resides 1–290) was expressed and purified as described (26). Purified recombinant VWF propeptide was a generous gift of Dr. H. C. Jolla, CA) and specific primers (Proligo, Paris, France) as follows: strand, 5’-TTCGGATCCTAGTGG-3’ and a stop codon) is inserted into the unique EcoRV restriction site, generating a PCR fragment with the forward primer 5’-TTCGGATCCTAGTGG-3’.

**TOPO. After sequence analysis, the BamHI-SrfI fragment was ligated into BamHI-EcoRV-digested pNUT-His. pNUT-VWF/A1-A3 was constructed by generating a PCR fragment with the forward primer 5’-CTGAGGCTCTTGGGATCCTAGTGG-3’, containing the unique BamHI restriction site (underlined). The PCR fragment was ligated into pCR2.1-

**Clearance of VWF in Mice**—VWF-deficient mice were injected intravenously with radiolabeled pd-VWF (5 μg/mouse, corresponding to 1–2 × 10^6 cpm/mouse) diluted in phosphate-buffered saline containing 3% albumin. At different time points (3, 15, and 30 min and 1, 2, and 4 h after injection) mice were anesthetized with trichloroethanol (0.15 ml per 10 g of body weight) and bled by retro-orbital venous plexus puncture. Subsequently, the mice were sacrificed, and various organs (heart, lungs, liver, spleen, stomach, kidneys and left upper leg) were collected, which were analyzed for the presence of radioactivity. Three mice were used per time point.

**Clearance of VWF in Mice**—VWF-deficient mice were injected intravenously with wt-VWF or variants thereof (5 μg/mouse) diluted in phosphate-buffered saline containing 3% bovine albumin. At different time points (3, 15, and 30 min and 24 h after injection) mice were anesthetized with trichloroethanol (0.15 ml per 10 g of body weight), blood was collected by eye bleed, and plasma was prepared as described (33). Three to six mice were used for each time point, and each mouse was bled only once. VWF antigen levels were quantified in a previously described immunosorbent assay using polyclonal antibodies (32). Normal pooled plasma from 40 healthy donors was used as a reference, assuming that 1 ml of pooled plasma contains 10 μg of VWF (35 nm, based on a monomeric molecular mass of 250 kDa). To quantify antigen values of VWF/D-A3 and VWF/D-D3, polyclonal rabbit antibodies directed against the D3-D3 region were used as capturing antibodies. In addition, a purified preparation of VWF/D-A3 was used as a reference.

**Surface Plasmon Resonance Analysis**—Surface plasmon resonance (SPR) binding assays were performed employing a Biacore2000 system (Biacore AB, Uppsala, Sweden). Collagen and GpIbα binding experiments were performed as described previously (26, 32). For FVIII light chain and VWF propeptide binding experiments, wt-VWF, VWF/ D2509G were purified from conditioned serum-free medium (Ultroser G from Biosepra, Cergy-Saint Christophe, France) by immunofinity chromatography employing the antibody RU-8 as described (25). pNUT-VWF/A1-CK, encoding residues 1260–1874 containing an amino-terminal His tag and was generously provided by Dr. S. Tsuji (University Medical Center Utrecht, The Netherlands). pNUT-VWF/A1-CK, encoding residues 1260–2813, was constructed by subcloning a Nhel-EcoRI fragment from pNUT-VWF into pNUT-VWF/A1-CK.

**Biodistribution of VWF**—VWF-deficient or wild-type mice were injected intravenously with radiolabeled pd-VWF (5 μg/mouse, corresponding to 1–2 × 10^6 cpm/mouse) diluted in phosphate-buffered saline containing 3% albumin. At different time points (3, 15, and 30 min and 24 h after injection) mice were anesthetized with trichloroethanol (0.15 ml per 10 g of body weight), blood was collected by eye bleed, and plasma was prepared as described (33). Three to six mice were used for each time point, and each mouse was bled only once. VWF antigen levels were quantified in a previously described immunosorbent assay using polyclonal antibodies (32). Normal pooled plasma from 40 healthy donors was used as a reference, assuming that 1 ml of pooled plasma contains 10 μg of VWF (35 nm, based on a monomeric molecular mass of 250 kDa). To quantify antigen values of VWF/D-A3 and VWF/D-D3, polyclonal rabbit antibodies directed against the D3-D3 region were used as capturing antibodies. In addition, a purified preparation of VWF/D-A3 was used as a reference.

**Clearance of VWF in Mice**—VWF-deficient mice were injected intravenously with wt-VWF or variants thereof (5 μg/mouse) diluted in phosphate-buffered saline containing 3% bovine albumin. At different time points (3, 15, and 30 min and 24 h after injection) mice were anesthetized with trichloroethanol (0.15 ml per 10 g of body weight), blood was collected by eye bleed, and plasma was prepared as described (33). Three to six mice were used for each time point, and each mouse was bled only once. VWF antigen levels were quantified in a previously described immunosorbent assay using polyclonal antibodies (32). Normal pooled plasma from 40 healthy donors was used as a reference, assuming that 1 ml of pooled plasma contains 10 μg of VWF (35 nm, based on a monomeric molecular mass of 250 kDa). To quantify antigen values of VWF/D-A3 and VWF/D-D3, polyclonal rabbit antibodies directed against the D3-D3 region were used as capturing antibodies. In addition, a purified preparation of VWF/D-A3 was used as a reference.
Clearance of VWF

These data indicate that intravenously administered VWF is primarily targeted to the liver.

Biphasic Clearance of Recombinant wt-VWF—To investigate the removal of VWF from plasma, VWF-deficient mice were injected intravenously with recombinant wt-VWF. At indicated time points after injection, mice were bled and plasma was analyzed for VWF antigen. The recovery appeared to be 79 ± 14% in samples obtained 3 min after injection (Table I).

Pharmacokinetic analysis allowed the calculation of the apparent half-lives, which were 12.6 ± 0.9 min and 3.0 ± 0.9 h for the rapid and slow phase, respectively. Furthermore, MRT was calculated to be 2.8 ± 0.7 h (Table I).

High and Low Molecular Weight Multimeric VWF Display Similar Survival in Mice—Preparations containing multimerized VWF are heterogeneous, as the number of included subunits may vary between molecules (from 2 to >20). To address whether the clearance of VWF depends on its extent of multimerization, we assessed the survival of pd-VWF fractions enriched in either high (predominantly 14-mer and higher) or low (predominantly dimers and tetramers) molecular weight multimers (see inset, Fig. 3) using VWF-deficient mice. These experiments revealed that both VWF preparations were cleared from plasma in a similar manner (Fig. 3). Indeed, similar pharmacokinetic parameters could be calculated from the experimental data (Table 1). Moreover, the clearance of both high and low molecular weight multimers was similar to that of recombinant wt-VWF. The possibility was considered that the clearance of larger multimers was similar to that of smaller multimers, because these large multimers were converted into smaller forms before removal from circulation. However, analysis of the plasma samples demonstrated that the large multimeric forms were not converted into smaller fragments before disappearance from the plasma (data not shown). Thus, the rate of VWF clearance is independent of its multimeric size in our experimental model.

Opposite Effects of Amino- and Carboxyl-terminal Truncations on the Plasma Survival of VWF—In view of the complex protein structure of VWF, it was of further interest to study the contribution of the various regions of the VWF molecule to its clearance. To this end, a number of recombinant VWF variants with amino- and/or carboxyl-terminal truncations were constructed (Fig. 4A). Purified proteins were administered to VWF-deficient mice by tail vein injection. With regard to a fragment consisting of the A1-A2-A3 domains selectively (i.e. VWF/A1-A3), a clearance pattern similar to wt-VWF was observed (Fig. 4B). As summarized in Table I, similar pharmacokinetic parameters were obtained for this variant and wt-VWF. In contrast, a variant with an amino-terminal truncation (designated VWF/A1-CK) displayed an initial recovery that was significantly lower than that observed for wt-VWF (43 ± 16% versus 79 ± 14% (p = 0.01) for VWF/A1-CK and wt-VWF, respectively). In addition, VWF/A1-CK was cleared from plasma in the initial phase more rapidly than wt-VWF (Fig. 4B). Indeed, both t1/2α and MRT were significantly reduced for the truncated protein compared with wt-VWF (Table I).

For the variants with a deletion of the carboxyl-terminal part of the protein, designated VWF/D’-A3 and VWF/D’-D3, the initial recovery was indistinguishable from that of wt-VWF. However, in the rapid phase of the clearance, both variants disappeared slower from plasma than wt-VWF (Fig. 4B). For instance, 13 ± 1% residual wt-VWF remained in plasma 1 h after injection, whereas almost 2-fold more antigen could be detected for VWF/D’-A3 (21 ± 1.6%; p = 0.0018) and VWF/
VWF-deficient mice were injected intravenously with wt-VWF (5 µg/mouse). Mice were bled at various time points after injection (from 3 min to 24 h), and plasma was prepared and analyzed for the presence of VWF antigen (33). Each mouse was bled once, and 3–6 mice were used for each time point. Residual VWF concentrations were plotted against time, and data were fitted to a biexponential equation to obtain various pharmacokinetic parameters, including MRT (see “Experimental Procedures”). Recovery represents the percentage of VWF present in plasma 3 min after injection relative to the amount injected. Data are presented as mean ± S.D. p values were calculated using Student’s unpaired t-test and represent comparison to wt-VWF.

| Recovery | MRT | $t_{1/2}$ | $t_{1/5}$ |
|----------|-----|----------|----------|
| wt-VWF   | 79 ± 14 | 2.8 ± 0.7 | 12.6 ± 0.9 | 3.0 ± 0.9 |
| LMW-VWF  | 70 ± 11 | 2.3 ± 0.7 | 15.2 ± 1.9 | 1.9 ± 0.4 |
| HMW-VWF  | 72 ± 6  | 3.0 ± 1.4 | 14.1 ± 1.9 | 2.6 ± 1.1 |
| VWF/A1-A3| 62 ± 8  | 2.9 ± 0.6 | 16.2 ± 4.2 | 2.8 ± 0.6 |
| VWF/A1-CK| 43 ± 14 | 1.6 ± 0.1 | 8.0 ± 0.5  | 1.5 ± 0.3 |
| VWF/D'/A3| 70 ± 12 | 4.5 ± 0.2 | 19.8 ± 2.9 | 4.5 ± 0.1 |
| VWF/D'/D3| 75 ± 3  | 5.9 ± 1.4 | 23.4 ± 8.7 | 6.5 ± 0.5 |
| VWF/R1205H| 97 ± 8  | 0.3 ± 0.1 | 7.6 ± 0.2  | 0.3 ± 0.03|
| VWF/D2509G| 82 ± 5  | 2.3 ± 0.5 | 12.0 ± 2.2 | 2.2 ± 0.2 |

D'-D3 (22.2 ± 1.3%; p = 0.0006). Calculation of the pharmacokinetic parameters revealed that both $t_{1/2}$,α and MRT were prolonged for the truncated proteins (Table I). Taken together, these data suggest the following. (i) Multiple areas may be involved in the direct interaction with components that mediate the removal of VWF from the circulation; and (ii) particular regions within the VWF molecule may play a regulatory role in its survival. One example hereof might be the D'-D3 region, which seems to protect VWF from premature clearance.

Replacement of Arg-1205 by His Results in an Increased Clearance Rate—The observation that the D'-D3 domain appears to be involved in the clearance of VWF may be of relevance with regard to VWF mutants with amino acid replacements in this region as found in a number of patients suffering from type 1 VWD. For instance, mutation of the D3-domain residue Arg-1205 to His has been suggested to modulate the survival of VWF in patients (19). To study the direct contribution of Arg-1205 to the clearance of VWF, recombinant VWF/R1205H was expressed in baby hamster kidney-furin cells. Analysis of the conditioned medium revealed a normal pattern of multimerization for this mutant (Fig. 5A). However, when the purified protein was injected intravenously in VWF-deficient mice, VWF/R1205H disappeared remarkably rapidly from the circulation (Fig. 5B). One hour after injection, 2 ± 0.1% was detected in plasma, compared with 13 ± 1% for wt-VWF (p < 0.0001). Data analysis showed that $t_{1/2}$,α and $t_{1/2}$,β as well as the MRT were significantly reduced compared with the values obtained for wt-VWF (Table I). In contrast, when we tested the in vivo survival of another VWF mutant, i.e. VWF/D2509G comprising a mutation in the RGD motif, it appeared that this mutant was cleared from plasma to the same extent as wt-VWF (Fig. 5; Table I). These data are compatible with the view that replacement of Arg-1205 by His specifically results in an accelerated clearance of VWF.

Replacement of Arg-1205 by His Leaves Binding to Collagen and GpIba Unaffected—The finding that replacement of Arg-1205 by His impairs the in vivo survival of VWF prompted us to study this mutant in more detail by testing a number of
functional parameters. First, the binding of purified wt-VWF and VWF/R1205H to human collagen type III and to a recombinant VWF-binding fragment of GpIb/H9251 (residues 1–290) in the presence of botrocetin was examined by SPR analysis. Binding isotherms using the response at equilibrium were used to calculate apparent affinity constants (K_D), which are summarized in Table II. The steady-state binding data revealed that wt-VWF and VWF/R1205H display similar affinity for GpIb/H9251 (K_D = 30 and 27 nM for wt-VWF and the mutant, respectively) and for collagen (K_D = 2.9 and 3 nM for wt-VWF and the mutant, respectively). Apparently, replacement of Arg-1205 by His leaves complex formation through its A1 (GpIb/H9251) and A3 (collagen) domains unaffected.

**Table II**

Affinity constants for the interactions between VWF and its ligands

| Ligand                  | wt-VWF | VWF/R1205H |
|-------------------------|--------|------------|
| Collagen type III       | 2.9 ± 0.4 | 3.0 ± 0.2 |
| GpIba*                  | 30 ± 8.5 | 26.9 ± 3.2 |
| FVIII light chain       | 7.9 ± 1.0 | 8.0 ± 1.2 |
| VWF propeptide         | 2.52 ± 0.04 | 2.27 ± 0.06 |
| pH 7.4                  | 0.49 ± 0.01 | 0.57 ± 0.03 |
| pH 5.8                  | 0.21 ± 0.01 | 0.44 ± 0.02 |
| pH 5.2                  | 0.11 ± 0.01 | 0.24 ± 0.01 |

*Values are nM.
*Values are μM.

(K_D = 30 and 27 nM for wt-VWF and the mutant, respectively) and for collagen (K_D = 2.9 and 3 nM for wt-VWF and the mutant, respectively). Apparently, replacement of Arg-1205 by His leaves complex formation through its A1 (GpIba) and A3 (collagen) domains unaffected.
Incubations were performed in 125 mM NaCl, 25 mM Hepes at pH 7.4 (line I) or pH 6.5 (line II), or in 125 mM NaCl, 25 mM (CH$_3$)$_2$AsO$_2$Na at pH 5.8 (line III) or pH 5.2 (line IV) at a flow rate of 10 µl/min for 4 min at 25 °C. Ligand solution was replaced with buffer to initiate dissociation. For both panels, the signal is indicated in resonance units (RU) and is corrected for aspecific binding to the VWF/A1-CK control channel, which was <5% of the wt-VWF- and VWF/R1205H-coated channels.

and 8 nm for wt-VWF and VWF/R1205H, respectively; Table II).

With regard to the VWF propeptide, an SPR-based assay was developed to study its interaction with VWF. VWF was immobilized (14 fmol/mm$^2$) and incubated with purified recombinant VWF propeptide (280 nM). The interaction was examined at various pH values (between pH 5.2 and 7.4), because the binding of the propeptide has been reported to be pH-dependent (8). Whereas binding at pH 7.4 was weak, binding of the propeptide to wt-VWF was markedly more prominent at lower pH values. Increased binding was mainly due to a dissociation rate constant that was reduced one order of magnitude at lower pH values. Analysis of steady state binding isotherms revealed that propeptide binds to wt-VWF with low affinity at pH 7.4 (Table II). Reduction of the pH increases the affinity by >20-fold ($K_D = 2.5$ and 0.1 µM at pH 7.4 and 5.2, respectively).

As for wt-VWF, a pH-dependent interaction with propeptide was also observed for VWF/R1205H (Fig. 6B). Moreover, similar association and dissociation curves were obtained for the mutant and wt-VWF at pH 7.4 and 6.5 (Fig. 6). As summarized in Table II, both proteins proved similar in propeptide binding in terms of affinity under these conditions (Table II). In contrast, a less pronounced increase in association of propeptide to VWF/R1205H was observed when pH was further decreased to pH 5.8 and 5.2 (Fig. 6B). Indeed, the calculated affinity constants were 2-fold higher for the mutant when compared with wt-VWF (pH 5.8, $K_D = 0.2$ versus 0.4 µM; pH 5.2, $K_D = 0.1$ µM versus 0.2 µM, for wt-VWF and VWF/R1205H, respectively). Apparently, replacement of Arg-1205 by His is associated with suboptimal binding of propeptide at low pH.

**DISCUSSION**

Elevated plasma concentrations of hemostatic proteins are often associated with an increased thrombotic risk, whereas reduced levels may result in a bleeding tendency. This indicates that steady state plasma levels of these proteins need to be tightly regulated. Plasma levels represent a balance between biosynthetic and catabolic pathways. With regard to VWF, its biosynthetic pathway has been well studied in the last two decades (17, 18), whereas relatively little is known concerning its clearance. In the present study we used VWF-deficient mice as a model to get more insight into the molecular basis of how VWF is cleared from the circulation.

Infusion studies employing radiolabeled VWF revealed that this protein was predominantly targeted to the liver, whereas little contribution of other organs (including kidneys) to the uptake of VWF was detected (Fig. 1). In time, accumulation of radioactivity was observed in the stomach. Similar delayed accumulation of radioactivity in the stomach has been described for other proteins as well, including annexin V and insulin growth factor-binding protein-3 (34, 35). In case of the latter, this accumulated radioactivity represents small degraded peptides that are excreted from the liver and reabsorbed by the stomach. Considering the time course by which the radioactivity appears in liver and stomach, it seems likely that a similar process is present for VWF. Whether VWF is taken up in the liver in a receptor-mediated manner is unclear from the present study. However, in view of its relatively large size and rapid accumulation in the liver, it seems conceivable that VWF is removed from the circulation by an active, receptor-dependent process.

We considered the possibility that the clearance of VWF is partially dependent on the presence of VWF in the circulation or extravascular space. However, a similar biodistribution was observed in normal and VWF-deficient mice in terms of organ distribution and time course (Fig. 1). VWF is cleared from plasma in a biphasic manner characterized by a rapid initial phase and a slow terminal phase (Table I). The MRT was calculated to be 2.8 h. In this respect, human VWF is cleared from the murine circulation to the same extent as reported previously for human VWF in rats (22). The half-lives of VWF in these rodents are considerably shorter compared with the half-life of VWF in humans, which is ~14 h (36, 37). Similar differences in the clearance rate between species have been reported for numerous other proteins, including annexin V and insulin growth factor IX. Whereas its half-life in humans is ~18 h (38), it is reduced 7-fold in mice (39). Probably, this reflects the basal metabolic rate, which is increased in mice.

One intriguing observation relates to the similar survival of high and low multimeric VWF preparations (Fig. 4), which suggests that the clearance of VWF is independent of its multimeric size in our experimental model. This observation is opposite to the findings that highly multimerized human VWF is cleared more rapidly than its low multimeric counterpart in rats as well as in dogs (22, 40). It might be that high molecular weight multimers of human VWF are converted into smaller multimers in dogs and rats. Such conversion could not be observed in our experiments, nor could we detect proteolytic degradation of VWF in murine plasma (data not shown). This is in line with the notion that human VWF is not proteolyzed by the murine ADAMTS-13 protease. Alternatively, high molecular weight human VWF may display enhanced binding to the platelet GpiIba/IX/V receptor in rats and dogs but not in mice. Indeed, it has been reported that human VWF is not recognized by murine GpiIba (41). This multimerization-independent sur-

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* C. V. Denis, unpublished observation.
It seems plausible that a receptor-recognition site is present in the A1-A3 region itself (Fig. 4D). Considering the clearance rate of the VWF/D4-CK region, we found that its absence is associated with a slower clearance (Fig. 4C), suggesting that deletion of these domains leads to the removal of a receptor-interactive site. However, when the D4-CK domains were linked to the A1-A3 region (i.e. deletion of the D3-D3 region), this protein was cleared more rapidly than the A1-A3 region itself (Fig. 4B; Table I). This points to a regulatory role of the D4-CK region by enhancing A1-A3 domain-mediated clearance. Our data do not allow us to distinguish between both possibilities, which are not mutually exclusive.

(iii) Considering the clearance rate of the VWF/D3 variant, it seems plausible that a receptor-recognition site is present in the D3-D3 region as well. Furthermore, the D3-A3 fragment (i.e. deletion of the D4-CK domains) is cleared less rapidly than the A1-A3 fragment, which could be compatible with the D3-D3 region serving a regulatory role by reducing the clearance rate.

The involvement of the D3-D3 region in VWF clearance is supported by a recent report by Casonato and co-workers (19). They observed that patients harboring an Arg-1205 to His mutation (44), which is absent in some (but not all) patients with the R1205H mutation (44–47).

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