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## Importance of Factors H and I for the Adherence of C3b-Coated Erythrocytes to Cells

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### Abstract

The role of cell membrane-associated human factor H for the binding of cell-bound C3b to complement receptor-carrying (CR<sup>+</sup>) cells was investigated. Pretreatment of CR<sup>+</sup> cells with antibodies to factor H inhibited the adherence of C3b-coated red cells to human tonsil lymphocytes (TL) and peripheral blood monocytes (Mø). The C3b receptor reactivity of human polymorphonuclear leucocytes (PMN) was not influenced and the one of Raji lymphoblastoid cells only slightly influenced; iC3b and C3d receptor reactivity was in no case affected.

When diisopropylfluorophosphate (DFP) in a concentration of 0.1 mM was present during pretreatment of the CR<sup>+</sup> cells with anti H, the antibodies gained the capacity to inhibit the adherence of C3b-coated erythrocytes to Raji cells; this effect was dose-dependent with respect to DFP. In contrast, there was no influence of DFP on the inhibition pattern of anti H in the case of TL and Mø. The adherence of C3b-coated erythrocytes to PMN remained unaffected by anti H antibodies in the presence of DFP.

Polyclonal as well as monoclonal antibodies directed against human factor I inhibited the binding of C3b cells to Raji cells but not to TL. Additionally, when anti I and anti H antibodies were both present, C3b receptor reactivity of Raji cells was inhibited to a larger extent than with either antibody alone; again, TL remained unaffected.

Results obtained by washing the Raji cells before and after treatment with anti H and anti I suggest that the respective antibodies act on factor H primarily on the level of the cell membrane and on factor I in the fluid phase.

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*Abbreviations:* C1, C2, C3, C4, C5 = first, second, third, fourth, fifth component of the complement system; C3b = b fragment of C3; iC3b = C3b treated with factors H and I; C3d = d fragment of C3; CR<sup>+</sup> = complement receptor carrying; CR<sup>-</sup> = complement receptor negative; DFP = diisopropylfluorophosphate; EAC14<sup>°</sup>23b = SRBC coated with antibody, C1, C4, oxydized C2 and C3; EAC14<sup>°</sup>23bi = EAC14<sup>°</sup>23b treated with factors H and I; EAC12<sup>°</sup>23d = EAC14<sup>°</sup>23bi treated with trypsin; gp = glycoprotein; H = factor H of the alternative pathway of complement activation; I = factor I of the alternative pathway of complement activation; MEM = minimum essential medium; MW = molecular weight; Mø = human peripheral blood monocytes; PBS = phosphate-buffered isotonic saline; PMN = polymorphonuclear leucocytes; PMW = poke wheat mitogen; PMW-TL = PMW-stimulated TL; SRBC = sheep red blood cells; TL = human tonsil lymphocytes; VBS-EDTA = veronal-buffered isotonic saline containing 20mM EDTA.

The results presented indicate that factor H may, to a certain extent, be involved in the binding process of particle-bound C3b to CR<sup>+</sup> cells. However, regarding lymphocytes and Raji cells, different mechanisms have to be proposed for the adherence of C3b-coated erythrocytes. In the case of Raji cells, we believe that membrane-associated proteases are involved in the binding of EAC14°23b to the CR<sup>+</sup> cell. It may be that a conversion of C3b to iC3b occurs after the initial adherence of C3b-coated erythrocytes to Raji cells.

## Introduction

During the last few years, research on receptors for the various cleavage products of C3 had made considerable progress: FEARON (1, 2) isolated from human erythrocytes a glycoprotein with a molecular weight of 205,000 dalton (gp 205) which was shown to be present also on B lymphocytes, monocytes, and polymorphonuclear leucocytes (PMN). DOBSON et al. (3) investigating human red cells also found a protein with a MW of 195,000 dalton, probably the same molecule as described by FEARON (1, 2). Antibodies directed against this molecule were shown to inhibit the adherence of C3b-coated erythrocytes to human red cells, B lymphocytes, monocytes and PMN (1, 2, 3, 4). For this reason, the gp 205 (1, 2) was assumed to represent the C3b receptor.

Beyond this, receptors for iC3b (5), for C3d (6), and factor H (7, 8, 9, 10) have been described. However, regarding the binding mechanisms of C3b to complement receptor carrying (CR<sup>+</sup>) cells, there are reports suggesting that, apart from the C3b receptor molecule described by FEARON (1, 2), additional molecules might represent binding sites for C3b: DIERICH et al. (11) could show that antibodies against  $\alpha_1$ -anti-trypsin inhibited C3b receptor reactivity on Raji B lymphoblastoid cells and on human erythrocytes. Furthermore, MUSSEL et al. (12) isolated from human red cells a glycoprotein (MW 55,000, gp 55) with C3b receptor reactivities. Monoclonal antibodies raised against gp 55 inhibited the adherence of C3b-coated sheep red blood cells (SRBC) to human red cells, Raji cells, and tonsil lymphocytes, but surprisingly not to monocytes and PMN (13). Solubilized Raji cell membranes seemed capable of regulating the activity of the alternative pathway of convertase, an effect distinct from that of factor H and gp 205 (14). This membrane preparation also revealed C3b receptor-like function (14). Cell membrane lysates from human erythrocytes and Raji cells, but not lysates from a CR<sup>-</sup> cell line, could bind to and modify C3b (15). Furthermore, BAREL et al. (16) isolated from Raji cell membranes by C3-dependent affinity chromatography a C3b receptor-like molecule with an apparent MW of 140,000 g/mol. These data point to the possibility that cell membrane-associated molecules other than gp 205 might act as binding sites for C3b. Since factor H reveals high affinity for C3b and since it was recently shown that H is a membrane constituent for B lymphocytes (17), we looked whether cell membrane-associated factor H could provide binding sites for cell-bound C3b.

## Materials and Methods

### *Buffers*

The following buffers were used: PBS, phosphate-buffered saline (140 mM NaCl, 10 mM Na-phosphate, pH 7.2); VBS-EDTA, veronal-buffered saline, containing EDTA (140 mM NaCl, 5 mM Na-5,5-diethylbarbiturate, 20 mM EDTA, 0.2% gelatine, pH 7.2).

### *Cells*

Raji cells were grown in RPMI 1640, supplemented with 10% heat-inactivated (1 h, 56 °C) fetal calf serum (FCS), glutamine (300 µg/ml), gentamicin (100 µg/ml) (Autopow Flow Laboratories, Bonn, FRG). The cells harvested from mid-log phase cultures ( $0.8\text{--}1.3 \times 10^6$  cells/ml) were washed three times with VBS and then adjusted to  $2 \times 10^6$  cells/ml VBS-EDTA. Human monocytes (Mø) and granulocytes (PMN) were obtained from 25 ml heparinized venous blood. After density gradient centrifugation on Ficoll Paque (Pharmacia, Freiburg, FRG) for 30 min at  $400 \times g$ , the cells from the interface (as a source of Mø) were collected, washed three times with PBS, adjusted to  $1.5\text{--}2.0 \times 10^6$  cells/ml and allowed to adhere (200 µl) to Lab-Tek tissue culture chambers (Miles Laboratories Inc., Naperville, Ill., USA) for 1 h at 4 °C. Then, non-adherent cells were decanted and the slides were gently dipped into cold VBS-EDTA. The pellet of the gradient centrifugation (red cells and PMN) was washed three times in PBS and then resuspended in 5 ml H<sub>2</sub>O gently shaken for 1 min. Thereafter, the mixture was diluted with the 10-fold volume of PBS. The procedure was repeated once or twice, according to the number of non-lysed red cells. Finally, the PMN were adjusted to  $2 \times 10^6$  cells/ml VBS-EDTA. Fresh human tonsils were minced and passed through a steel mesh; the cells were layered onto Ficoll Paque and centrifuged for 30 min at  $400 \times g$ . The cells from the interface were washed three times with PBS and then adjusted to  $2 \times 10^6$  cells/ml VBS-EDTA. For stimulation, cells from the interface were washed three times in minimum essential medium (MEM) (Flow). 1 ml cell suspension ( $1 \times 10^6$ /ml) was cultured in flat bottom wells with MEM containing 2.5% human-heat-inactivated AB serum, 7.5% FCS, 100 µg gentamicin/ml, and 80 µg pokeweed mitogen (PWM)/ml for 72 h at 37 °C. Thereafter, the cells were washed three times in MEM and then adjusted to  $2 \times 10^6$  cells/ml in VBS-EDTA (PWM-TL).

### *Intermediates*

Sheep erythrocytes (SRBC) coated with antibodies, C1, C4, oxydized C2, and C3b (EAC14°23b) were prepared as described (18). Uptake of C3 was quantitated using labeled C3 (18). SRBC coated with  $2 \times 10^4$  C3 molecules/cell (for rosette formation with TL) and  $8 \times 10^4$ /cell (Raji cells) were used. Conversion of EAC14°23b to EAC14°23bi and EAC14°23d was described in detail (18). The C3 used for these experiments was purified essentially according to Hammer et al. (19). It should be mentioned that this C3 preparation was free of H and C5. The C3 was tested for H with a hemagglutination assay (sensitivity 50 pg/ml) (20); for detection of C5, the final C3 preparation was concentrated ten-fold and the standard hemolytic assay was performed (21). No C5 activity could be found. Factor I was prepared as described (22).

### *Antibodies*

Goat F(ab')<sub>2</sub> anti H were prepared as described previously (9). These antibody fragments were further purified by affinity chromatography on H-coated Sepharose 4B (Pharmacia) (2 mg H/ml Sepharose 4B). 5 ml of the gel was equilibrated with PBS and 5 ml antibody solution (0.5 mg/ml PBS) was processed over the column (1 × 10 cm). The gel was washed extensively with PBS and then with 5 ml 3M urea; no anti-H activity was eluted. The antibodies were eluted from the gel with 3M KSCN, then dialyzed against PBS and adjusted to 0.5 mg/ml. Antibodies directed against human factor I were raised by immunization of rabbits. The IgG fraction was prepared by chromatography on DEAE-Sephadex (Pharmacia). The break-

through fraction was dialyzed against PBS (dilution with respect to whole serum 1:5). Monoclonal antibodies against human factor I (ascites fluid) were a generous gift from Dr. L.-M. Hsiung from Oxford, U.K. (23).

#### *Complement receptor assay*

20  $\mu$ l of the respective complement receptor carrying (CR<sup>+</sup>) cells ( $2 \times 10^6$ /ml) except monocytes were incubated in a microtiter plate with 20  $\mu$ l VBS-EDTA or 20  $\mu$ l antibody dilution for 30 min at room temperature. Thereafter, 20  $\mu$ l intermediates ( $1.3 \times 10^8$ /ml VBS-EDTA) were added and the mixture was incubated for 30 min at 37°C, and rosette formation was evaluated immediately thereafter. Alternatively, after incubation with the antibody solution 200  $\mu$ l VBS-EDTA was added to each well and the plate was centrifuged for 5 min at  $250 \times g$  and the supernatant aspirated. 20  $\mu$ l VBS-EDTA and 20  $\mu$ l intermediates were added, and the mixture was incubated for 30 min at 37°C. For some experiments, Raji cells were incubated for 30 min at room temperature with 20  $\mu$ l antibody solution of the respective dilution, next 20  $\mu$ l EAC14°23b were added, and the mixture was incubated for 30 min at 27°C. In case of Raji cells, after rosette formation with EAC14°23b, the cells were treated with glutaraldehyde (final concentration 0.06%) to prevent the decay of the formed rosettes (24). For rosette formation with monocytes, adherent cells were incubated on Lab-Tek chambers with 200  $\mu$ l VBS-EDTA or 200  $\mu$ l antibody solution for 30 min at 4°C. The cells were then washed carefully with VBS-EDTA. Subsequently, 100  $\mu$ l of the respective intermediate suspension ( $1.3 \times 10^8$ /ml) were layered onto the slide. After incubation (15 min, 37°C), the slides were dipped into VBS-EDTA containing 0.02% NaN<sub>3</sub> to remove unbound erythrocytes. Rosette formation was evaluated immediately thereafter. In some experiments, diisopropylfluorophosphate (DFP), Merck (Darmstadt, FRG), was present during preincubation of the CR<sup>+</sup> cells with the anti H antibodies.

#### *Immune adherence assay*

This test was performed as described in detail previously (13). Briefly, 20  $\mu$ l of 0.25–0.5% suspension of human red cells (blood group 0<sup>+</sup>) were incubated with 20  $\mu$ l VBS-EDTA of antibody solution for 30 min at room temperature. 20  $\mu$ l of EAC14°23b were added and the mixture was incubated for 30 min at 37°C. The agglutination pattern was evaluated 30–60 min thereafter.

## Results

### *Influence of pretreatment of CR<sup>+</sup> cells with F(ab')<sub>2</sub> anti-H on rosette formation with C3-coated SRBC*

Pretreatment of TL, Mø, and PWM-TL with F(ab')<sub>2</sub> anti-H resulted in a dose-dependent loss of the capacity of these cells to form rosettes with EAC14°23b (Fig. 1 A, B, C). In contrast, anti-H had no or only slight inhibitory effects on PMN and Raji cells, respectively (Fig. 1 C, D). iC3b- and C3d-dependent rosette formation was in no instance influenced by H antibodies (Fig. 1).

F(ab')<sub>2</sub> anti-H exhibited no influence on the immune adherence reaction (dilutions tested 1:4–1:5096).

### *Influence of DFP on the anti-H-dependent inhibition of rosette formation with EAC14°23b*

In earlier experiments we observed that DFP modified C3b-dependent rosette formation on activated lymphocytes and on Raji cells (24). There-

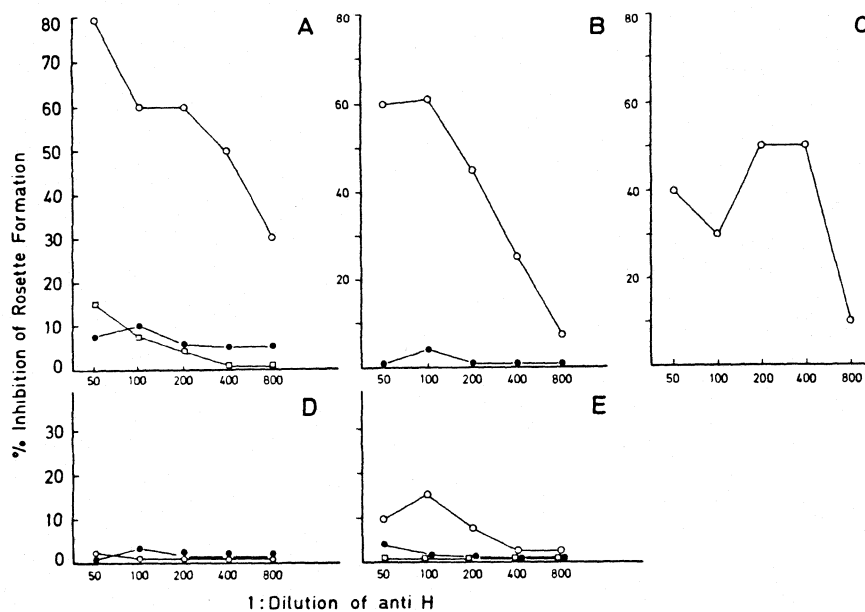


Fig. 1. Inhibition of rosette formation between tonsil lymphocytes (A), Mø (B), PWM-stimulated lymphocytes (C), PMN (D), and Raji cells (E), respectively, and EAC14°23b (○—○), EAC14°23bi (●—●), and EAC14°23d (□—□) with F(ab')<sub>2</sub> anti-H.

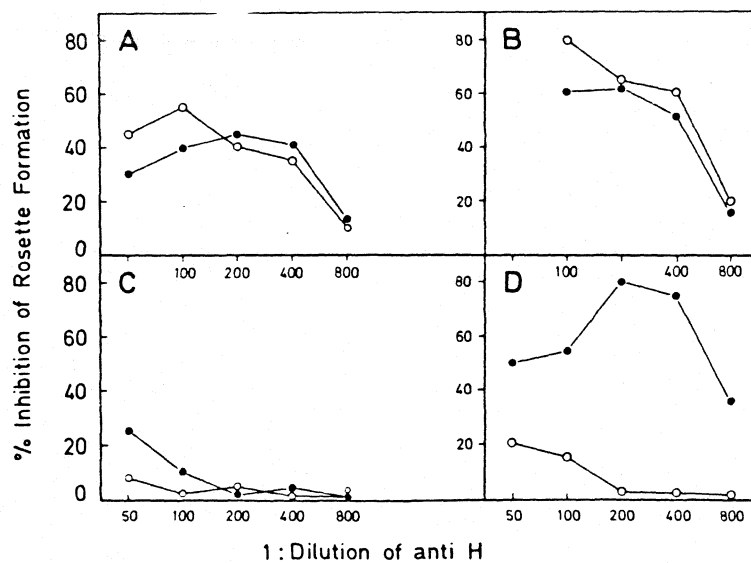


Fig. 2. Influence of DFP (0.1 mM) on the F(ab')<sub>2</sub> anti-H-mediated inhibition of the rosette formation between tonsil lymphocytes (A), Mø (B), PMN (C), and Raji cells (D), respectively, and EAC14°23b. Buffer ○—○, 0.1 mM DFP ●—●.

Table 1. Influence of DFP and F (ab')<sub>2</sub> anti-H on the rosette formation of Raji cells with EAC14°23b

Dilution of anti-H	Concentration of DFP (mM)			
	0	0.1	0.2	1
1:100	0 <sup>a</sup>	49	15	0
1:200	0	85	45	5
1:400	0	60	18	20
1:800	0	25	5	0
buffer control	31 % <sup>b</sup>	39 %	38 %	28 %

Raji cells were incubated with anti-H and DFP (final concentrations indicated), both at varying concentrations, and then washed

<sup>a</sup> % inhibition of rosette formation

<sup>b</sup> % rosette forming cells

fore, we examined the influence of DFP (0.1 mM) on the anti-H-mediated inhibition of the adherence of EAC14°23b to CR<sup>+</sup> cells. The inhibition pattern of anti-H remained apparently unaffected by 0.1 mM DFP in the case of TL and Mø (Fig. 2, A, B). The C3b-dependent rosette formation of PMN could not be inhibited with anti-H neither in the presence nor in the absence of DFP (0.1 mM) (Fig. 2C). In the case of Raji cells, however, anti-H antibodies gained the capacity to inhibit the rosette formation with EAC14°23b in the presence of 0.1 mM DFP (Fig. 2D). Table 1 shows that this effect was dose-dependent with respect to DFP and anti-H. The best DFP-dependent enhancement of the anti-H mediated inhibition of the C3b-dependent rosette formation of Raji cells was observed with 0.1 mM DFP, whereas, 0.2 mM DFP was less effective and 1 mM DFP had nearly no effect.

#### *Effect of antibodies against human factor I on the rosette formation of CR<sup>+</sup> cells with EAC14°23b*

Fig. 3 shows the effect of polyclonal antibodies to I on the C3b-dependent rosette formation in the presence of anti-H at various concentrations. The antibodies to factor I did not influence the anti-H-mediated inhibition of the rosette formation between TL and C3b cells (Fig. 3B). In contrast, using Raji cells as CR<sup>+</sup> cells, it could be observed that antibodies against H gained the capacity to inhibit the adherence of C3b cells in the presence of anti-I antibodies (Fig. 3A). By themselves, these antibodies exhibited only a slight inhibitory capacity (40 % reduced to 34 % rosette formation). For preincubation of the Raji cells and the TL, 20 µl of the undiluted anti-I IgG fraction were used. However, when a higher volume (100 µl) of this IgG fraction was applied, a dose-dependent inhibition of the C3b-dependent rosette formation of Raji could be noticed (Fig. 4).

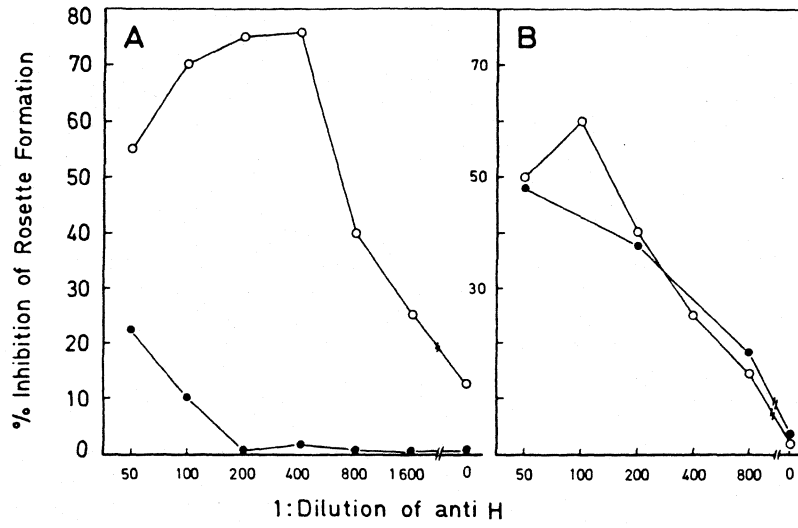


Fig. 3. Influence of anti C3b-INA (anti-I) on the anti-H-mediated inhibition of the rosette formation between Raji cells (A), tonsil lymphocytes (B), respectively, and EAC14°23b. Buffer ●—●, 20 µl anti-I (conc) ○—○.

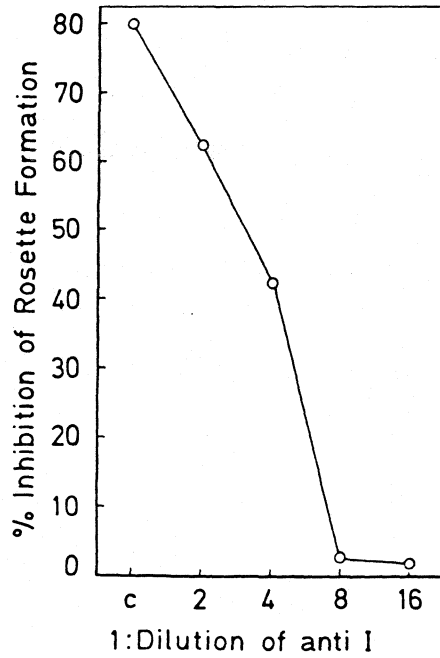


Fig. 4. Influence of anti-I on the anti-H-mediated inhibition of the rosette formation between Raji cells and EAC14°23b. Assay: 20 µl Raji cells ( $2 \times 10^6$ ), 100 µl anti-I, ○—○.

Table 2. Influence of anti-I monoclonal antibodies and F(ab')<sub>2</sub> anti-H on the rosette formation of Raji cells with EAC14°23b

Dilution of anti-I	anti-H					
	0	1:50	1:100	1:200	1:400	1:800
1:100	20 <sup>a</sup>	0	15	31	17	17
1:500	61	n.d.	n.d.	n.d.	n.d.	n.d.
1:2,500	50	70	81	52	31	33
1:10,000	23	36	33	73	63	43
1:25,000	13	n.d.	n.d.	n.d.	n.d.	n.d.
1:100,000	5	22	15	13	23	8
buffer control	0 <sup>b</sup>	3	10	15	8	5

<sup>a</sup> % inhibition of rosette formation

<sup>b</sup> buffer control 48 % rosette-forming cells (= 0 % inhibition)  
(mean of three experiments)

The results obtained with polyclonal IgG anti factor I were substantiated by experiments using anti-I monoclonal antibodies that were prepared and characterized recently by HSIUNG et al. (23). The data summarized in Table 2 are in accordance with those obtained with polyclonal antibodies.

In contrast to these observations with Raji cells, C3b-dependent rosette formation of TL was unaffected by monoclonal antibodies against factor I (Table 3).

#### *Effect of transient treatment of CR<sup>+</sup> cells with anti-H and anti-I*

Washing of the CR<sup>+</sup> cells after treatment with anti-H, and before using them in the rosette assay, did not reduce or influence the inhibitory capacity of anti-H. The results corresponded to those seen in Fig. 1. For anti-I antibodies, the results turned out to be different. Washing of the cells after incubation with these antibodies considerably affected their capacity to enhance the anti-H-dependent inhibition of the rosette formation of Raji

Table 3. Influence of anti-I monoclonal antibodies on the rosette formation of tonsil lymphocytes with EAC14°23b

anti I	
1:50	25 <sup>a</sup>
1:250	3
1:750	8
1:3,750	5
0	63 <sup>b</sup>

<sup>a</sup> % inhibition of rosette formation

<sup>b</sup> % rosette forming cells

Table 4. Influence of washing after incubation of Raji cells with F(ab')<sub>2</sub> anti-H and anti-I on rosette formation with EAC14°23b

	Dilution of F(ab') <sub>2</sub> anti-H			Washing procedure
	1:100	1:200	buffer	
A. no anti-I present	13	0	0	Raji cells incubated with F(ab') <sub>2</sub> anti-H, anti-I, buffer; EAC14°23b added thereafter.
20 µl of anti-I present	66	77	63	
B. no anti-I present	17	23	0	Raji cells incubated with F(ab') <sub>2</sub> anti-H, anti-I, buffer and then washed; EAC14°23b added thereafter.
20 µl of anti-I present	50	55	21	
C. 20 µl of anti-I present	89	83	45	Raji cells incubated with F(ab') <sub>2</sub> anti-H or buffer and then washed; thereafter, anti-I and EAC14°23b added.

Numbers given represent % rosette inhibition. The percentage of rosette-forming cells in the control [no anti-I, no F(ab')<sub>2</sub> ranged from 50–60 %].

cells with C3b cells (Table 4). The data shown in Table 4 indicate the best inhibition of the adherence of C3b-coated SRBC to Raji cells when the CR<sup>+</sup> cells were preincubated with anti-H, then washed and afterwards incubated simultaneously with anti-I and EAC14°23b (Table 4C). The weakest inhibition was seen when the Raji cells were incubated with anti-H and anti-I, then washed and incubated with EAC14°23b (Table 4B).

## Discussion

Factor H, one of the six proteins of the alternative pathway of complement activation (25), is a well characterized protein (26, 27) that regulates the hemolytic cascade by binding to C3b (28) in competition to factor B (27). It represents an absolute requirement for the conversion of C3b to iC3b by factor I (22). Recently, by use of the sensitive direct antiglobulin rosetting reaction (DARR, 17), evidence was provided that H is associated with the lymphocyte membrane, although it cannot be concluded from these data whether this protein is synthesized by the cell, taken up from the serum and bound loosely, or attached to the lymphocytes via specific binding sites. In any case, our data support the finding that H is associated with the membrane and suggest that this membrane-associated H is involved in binding of cell-bound C3b: firstly, F(ab')<sub>2</sub> anti-H fragments reveal the capacity to inhibit the adherence of C3b-coated, but not of iC3b- and C3d-coated SRBC to tonsil lymphocytes and monocytes (Fig. 1A, B,

C); secondly, the inhibitory effect of treatment of CR<sup>+</sup> cells with anti-H was stable even if the cells were washed after the treatment and before contact with C3b carrying cells. Interestingly, C3-dependent rosetting of PMN was not influenced by pretreating those cells with anti-H antibodies (Fig. 1D).

The mechanisms by which membrane-associated H affected C3-dependent binding is unclear. Firstly, membrane-associated H might represent the C3b receptor itself. Secondly, it might be located very close to the C3b receptor proper and when coated with anti H, sterically inhibit access to this receptor. Finally, H could enhance C3b-dependent rosette formation by acting as a modulator of C3b, thereby, tightening the contact to the receptor molecule. In case the adherence of C3b cells to the CR<sup>+</sup> cells would occur by direct binding to H, the role of the gp 205 molecule would be questionable. However, it has to be stressed that treatment of the lymphocytes and monocytes with anti-H led to an inhibition of only up to 60–80 % (Fig. 1A, B) while, on the other hand, antibodies to gp 205 were capable of completely blocking the binding of C3b cells (2, 3, 4). Based on these observations, we favour the second and third possibility.

With respect to Raji cells, the mechanism(s) of the binding of EAC14°23b to the surface of the cells seem to be even more complex: DFP, in a concentration of 0.1 mM showed a striking enhancement of the efficacy of the anti-H antibodies to inhibit the C3b-dependent rosette formation (Fig. 2D); no marked effect of preincubation of the CR<sup>+</sup> cells with DFP and anti-H was seen with lymphocytes, Mø and PMN (Fig. 2A, B, C). The most efficient concentration tested was found to be 0.1 mM, whereas higher concentrations were less (0.2 mM) or virtually ineffective (1 mM) in enhancing the anti-H dependent inhibition of the C3b-dependent rosette formation.

The observation that lower concentrations of DFP (0.1 mM) proved to be more effective than higher ones in enhancing the anti-H-mediated inhibition of the C3b-dependent rosette formation of Raji cells, point to a sensitive, possibly protease-controlled, equilibrium of reactions influencing membrane-associated proteins that are responsible for the adherence and binding of EAC14°23b. These findings fit well to data recently published (24): it was shown that DFP – present during rosette formation – enhanced the percentage of rosette forming Raji cells most effectively with a concentration of 0.1 mM. Higher concentrations (0.2–0.5 mM) were less effective or rather inhibitory (1 mM) for the C3b-dependent rosette formation of Raji cells (24). The Raji cells shared this feature with PWM-stimulated tonsil lymphocytes, but not – again in parallel to the data presented in this paper – with unstimulated small tonsil lymphocytes (24). These findings and the data presented in this paper suggest that the adherence of EAC14°23b to the cell surface (i.e. the «C3b receptor reactivity») is rather a metabolic event than a simple binding of a ligand to its receptor molecule, in particular since in the case of EAC14°23b, the C3b is presented to the

plasma membrane of the CR<sup>+</sup> cell in cluster-form. Distinct events on the cell surface of different cell types following the binding of EAC14°23b might be an explanation why the C3b receptor reactivity of different cell types was distinctly modulated by anti-H.

The results obtained from experiments with antibodies directed against human factor I suggest that different mechanisms have to be proposed for the adherence of EAC14°23b to normal lymphocytes and to Raji cells. In the case of normal lymphocytes, antibodies to I exhibited no influence on the rosette formation with EAC14°23b (Table 3); additionally, these antibodies did not enhance the anti-H-dependent inhibition of the C3b-dependent rosette formation. However, in the case of Raji cells, anti-I antibodies were active in both respects (Fig. 3, 4; Table 2). The latter observation obviously points to the assumption that on EAC14°23b the C3b was converted to iC3b during the rosette formation.

As shown in Table 4, washing reduced the effect of anti-I antibody on C3b-dependent rosette formation. Therefore, one could either assume that factor I involved in rosette formation is not membrane-associated (as would be factor H) or that it is very easily shed from the membrane. Possibly, C3b close enough in the vicinity of membrane-associated H is converted to iC3b by the action of (fluid phase?) factor I, thereby tightening the contact to the cell surface in an unknown way. Alternatively, the generated iC3b might lose the contact to the C3b receptors and rebind to iC3b receptors. However, whatever mechanism is operating, from published data (13-16, 24) and from data presented in this paper, it has to be assumed that the first step of adherence of C3b to the surface of Raji is mediated through a C3b-binding molecule: C3b-binding entities were isolated from Raji cell membranes (12, 14, 16) and antibodies raised against gp 55, a C3b-binding glycoprotein (12), inhibited the adherence of C3b-coated erythrocytes to Raji cells as well as to lymphocytes, red cells and glomerular cells (13). The initial interaction of C3b with the Raji cell membrane might be so weak that conversion to iC3b might be necessary to stabilize the contact between EAC14°23b and Raji cells. Possibly, factor I is released from Raji cells in response to triggering of membrane-associated H or of other possible C3b-binding sites. The mechanisms of this release of factor I should be distinct from that described by LAMBRIS et al. (8), which takes place in response to triggering (H-) receptors, and which can be inhibited by EDTA. The experiments described in this paper, however, were performed in the presence of 20 mM EDTA.

Although we observed the dependency of rosette formation on factor I only in the case of Raji lymphoblastoid cells, at present we do not want to exclude that on normal lymphocytes, too, a similar conversion process might take place during rosette formation. However, if there is such a conversion process in normal lymphocytes it would not be essential for the rosette formation. Probably, the C3b would be bound to its receptor with high affinity so that conversion of C3b to iC3b with eventual subsequent

binding to an iC3b receptor would not be essential for a stable binding of C3b to C3R<sup>+</sup> cells.

Staining of Raji cells, lymphocytes and phagocytic cells with commercially available FITC-labelled anti-H (9) resulted in a faint fluorescence. When using a double layer technique (goat anti-H and FITC-anti goat IgG) also only a faint staining of these cells was observed; additionally, in the case of Raji cells (~ 10%) and tonsil lymphocytes (~ 15%), small fluorescent patches could be seen (data not shown). To get more reliable results, experiments are under way to establish an ELISA technique, using whole cells or membrane preparations and monoclonal antibodies against factor H.

As shown in a previous paper, treatment of lymphocytic cells and PMN, but not of Mø with soluble factor H, improved the capacity of these cells to form rosettes with EAC14°23b (9). We think that in the case of receptor-bound factor H, probably different mechanisms for the binding of particle-bound C3b to CR<sup>+</sup> cells are working. Nevertheless, these experiments enhance the assumption that cell membrane-bound H can provide binding sites for C3b.

We feel that the data presented clearly indicate that factor H is involved in the adherence of EAC14°23b to tonsil lymphocytes, monocytes and Raji cells. The role of H for C3b-dependent rosette formation is obviously distinct from that of gp 205 (2, 3), since H seems to be important only for the binding of C3b-coated red cells to mononuclear cells but not to PMN and erythrocytes, whereas, on the other hand, all these cells, but not Raji cells (3), however, express gp 205 on their cell surface. Experiments are under way to clarify the mechanism(s), particularly in the case of Raji cells, by which H (and I) facilitates adherence of EAC14°23b.

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