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INTERACTION OF RHEUMATOID FACTOR AND ENTAMOEBA HISTOLYTICA

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SUMMARY

The amoebae's cytotoxicity test and the amoebae's lysis test were used to show possible interactions between rheumatoid factor (RF) and **Entamoeba histolytica**. Amoebae's cytotoxic activity (ACA) was inhibited by affinity chromatography purified antiamoebae rabbit IgG (RIgG). Enhanced inhibition could be demonstrated with RIgG plus RF. But the same marked inhibition of ACA could be seen when replacing RF by heat inactivated normal human serum as a control.

About 50% amoebae's lysis occurred when amoebae were brought together with native normal human serum (NNHS) as a source of complement. Amoebae's lysis increased to 60% when incubated with NHS plus human antiamoebae antibodies. No further augmentation could be obtained by the addition of RF. Using RIgG instead of human antibodies the lysis rate did not increase. Incubation of amoebae, NNHS, RIgG and RF even reduced amoebae's lysis. RF neither has an effect on ACA nor on complement mediated AL **in vitro**.

KEY WORDS: Entamoeba histolytica; Amoebae's lysis; Rheumatoid factor.

INTRODUCTION

Rheumatoid factors (RF) are mostly IgM antibodies directed to various antigenic determinants of the Fc part of autologous or homologous IgG. The occurrence of IgM with RF like activity has not only been reported in collagen vascular diseases like rheumatoid arthritis, but also in numerous infectious diseases, like endocarditis[2] and parasitic infections like malaria, trypanosomiasis, leishmaniasis, schistosomiasis and onchocerciasis[3,11,12,16,17,22]. The large number of infectious disease states known to be associated with rheumatoid factor production could be taken as evidence that this may be a normal immune response engendered in the host during many reversible and chronic infectious diseases[9].

CLARKSON & MELLOW[4] reported on RF acting in a protective way by showing that RF protects uninfected rat pups and dams from **Trypanosoma lewisii** infections. GREEN & PACKER[10] reported an inhibitory effect of RF on **Plasmodium falciparum** schizonts **in vitro**.

To our knowledge no studies exist dealing with the interaction of amoebae and RF.

In this work we investigated the **in vitro** interaction of **E. histolytica** with antiamoebae antibodies, human complement and rheumatoid factor.

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METHODS

Amoebae:

For all tests 72 hours cultured **E. histolytica** strain SFL 3 were taken. Modified TY-S-33 Medium was used[6,7,8]. Trophozoites were harvested and washed twice in RPM 1640 medium plus 10% fetal calf serum (RPMI/ FCS). Then amoebae were adjusted to the wanted cell number per ml.

Target cells:

K 562, a human erythroleukemia cell line was used as target for amoebae's cytotoxicity. K 562 cells were cultured in RPMI/ FCS.

Rheumatoid Factor (RF):

The source of RF was serum from a patient with inactive rheumatoid arthritis. The titer of RF was 1:1280 as determined by agglutination of Latex particles coated with human IgG (**Behring**) and 1:20.000 as measured by haemagglutination of erythrocytes coated with rabbit IgG (Raha-Test, **Fujirebio**). The serum was used after heat inactivation and absorption of IgG by Protein A (**Pansorbin, Behring**) and centrifugation. The titer after absorption was 1:5000 as measured by haemagglutination.

Human serum:

Freshly drawn normal human serum (NHS) was used which was negative with respect to all amoeba-specific serological tests (IIFT, IHA, ELISA). The complement activity was 36 $CH_{50}U/ml$.

One part of the same serum was heat inactivated at 56°C for 30 minutes (HNHS) and treated in the same way as the RF-containing serum.

Antibodies:

For preparation of antiamoebae antibodies, 30 ml of the serum from a patient suffering from an amoebic liver abscess was precipitated with a 33% $(NH_4)_2SO_4$ solution, leaving the IgG-fraction in solution. The IgG fraction was dialysed against isotonic phosphate-buffered saline, pH-7.4. As shown by immunoelectrophoresis, this preparation proved to be pure IgG (HIgG) and showed a highly positive reaction in the ELISA[28]

For preparation of rabbit antiamoebae antibodies, we immunized rabbits intramuscularly with amoebic antigens. The immunization schedule was week 0, 1, 2, 6 and a boosting dose 3 months later. Blood was drawn by intracardial punction.

For antigen preparation we took SFL 3 amoebae and homogenized the cells with the Potter-Elvejlham homogenizer at O°C. The resulting solution we mixed for the first immunizing dose with complete **Freund**'s adjuvant, for the next doses with incomplete **Freund**'s adjuvant.

The rabbit sera were test by the ELISA technique for antiamoebic activity[21]. All four sera showed positive reactions and were pooled. For getting pure IgG out of sera the 33% $(NH_4)_2SO_4$ precipitation method was used and proved by immunoelectrophoresis after dialysis against PBS.

AFFINITY CHROMATOGRAPHY

10^9 SFL 3 trophozoites were washed three times in PBS containing 2 mmol/ℓ phenylmethylsulfonylfluoride (PMSF), 10 mmol/ℓ ethylendiaminotetraacetate (EDTA) and 10 mmol/ℓ E-aminocaproicacid (EACA) and resuspended in this buffer at 4°C. Amoebae were homogenized by use of a Potter-Elvejlham homogenizer at 4°C. Subsequently, the homogenate was centrifuged at 1.500g for 20 minutes at 4°C. Then the supernatant was subjected to centrifugation at 25.000g for one hour at 4°C. The pellet (membrane fraction) was resuspended in coupling buffer and coupled to CNBr activated Sepharose 4 B (**Pharmacia Fine Chemicals**, Vienna, Austria) according to the manufacturer's order i.a. 10 ml rabbit IgG (20 mg/ml) were processed over the affinity column. After that the gel was washed by PBS until the base line at the UV monitoring system was reached. The amoebae specific antibodies were eluted with 0.1 mol/ℓ CH_3COOH, pH-2.5. After elution, the antibody preparation (RIgG) was immediately neutralised and dialysed against several changes of PBS. In the ELISA we could show a 30 fold enrichment of antiamoebae antibodies in comparison with IgG before affinity cromatography.

Amoebae's lysis test (AL)

Amoebae's lysis test was performed as described earlier[21,27]. Amoebae were labelled with ^{51}Cr. Then 5 x 10^5 amoebae were mixed with human serum, RF, antibodies and RPMI/FCS and incubated at 37°C for 30 minutes. Each test was carried out six fold.

Amoebae's cytotoxic activity test (ACA)

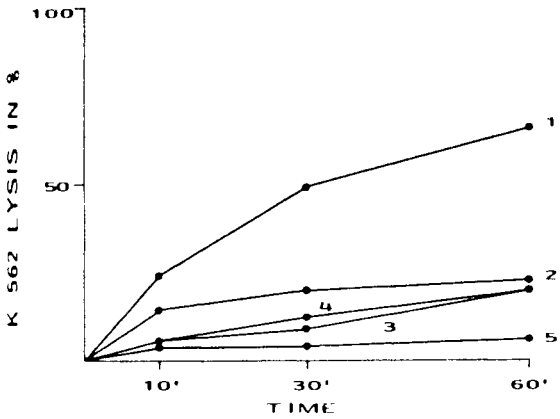
A modified microcytotoxicity assay was performed as described for K and NK lymphocytes[26]. K 562 target cells were labelled with ^{51}Cr. The effector to target cell ratio was adjusted to 5:1. 100 µl amoebae (washed twice in RPMI/FCS in a concentration of 5 x 10^5/ml) and 300 µl of RPMI/FCS or antibody or human serum or heat inactivated human serum or RF were mixed and incubated at 37°C for 30 minutes. Then 200 µl K 562 (5 x 10^4/ml in RPMI/FCS) were added. Tubes were centrifuged at 200g for 2 minutes and incubated at 37°C for 10, 30 and 60 minutes. The assay was performed in triplicates. Target cell lysis was calculated from the radioactivity released into the supernatant. As a control K 562 were incubated with RPMI/FCS instead of amoebae (spontaneous lysis).

RESULTS

Amoebae's cytotoxic activity (ACA) against target cells was inhibited by antiamoebae antibodies. **Figure 1** shows the

Figure 1

Inhibition of amoebae's cytotoxic activity by antibodies.



1 = SFL3 amoebae + K562
2 = SFL3 amoebae + K562 + RIgG
3 = SFL3 amoebae + K562 + RIgG + RF
4 = SFL3 amoebae + K562 + RIgG + HNHS
5 = K562 (spontaneous lysis)

TABLE 1

Amoebae's Lysis (AL) with human serum, rheumatoid factor and rabbit IgG after 30' incubation at 37°C

(% lysis)

EXPERIMENTS	I	II	III	IV
Samples No.	Am + RIgG + + RF + NNHS	Am + RIgG + + RPMI + NNHS	Am + RPMI + + RPMI + NNHS	Am + RPMI + + RPMI + RPMI
1	35.7	54.9	52.9	6.9
2	36.8	51.1	50.6	6.7
3	37.6	52.5	51.5	7.2
4	37.5	51.1	51.9	6.9
5	37.3	53.6	51.5	7.0
6	37.4	51.8	51.7	6.8
Mean	37.1	52.5	51.7	6.9
Median	37.3	52.2	51.6	6.9
Min-Max.	35.7-37.6	51.1-54.9	50.6-52.9	6.6-7.1

Am (1 ml) = 5 x 10^5 SFL3 amoebae/ml.
RF (1 ml) = RF containing heat inactivated human serum.
RPMI (1 ml) = RPMI 1640 + 10% FCS
RIgG (1 ml) = Rabbit IgG, antiamoebae antibodies affinity chromatographic purified, 100 mcg/ml.

NNHS (1 ml) = Native normal human serum.

TABLE 2

Amoebae's Lysis (AL) with human serum, rheumatoid factor and human IgG after 30' incubation at 37°C

(% lysis)

EXPERIMENTS	I	II	III	IV
Samples No.	Am + HIgG + + RF + NNHS	Am + HIgG + + RPMI + NNHS	Am + RPMI + + RPMI + NNHS	Am + RPMI + + RPMI + RPMI
1	61.2	61.2	54.2	6.6
2	62.8	61.7	54.6	6.8
3	61.4	61.3	55.6	7.1
4	60.8	62.3	55.0	7.0
5	61.1	62.1	54.6	6.7
6	63.5	62.3	54.8	6.7
Mean	61.8	61.8	54.8	6.8
Median	61.3	61.9	54.7	6.8
Min-Max.	60.8-63.5	61.2-62.3	54.2-55.6	6.7-7.2

Am (1 ml) = 5 x 10^5 SFL3 amoebae/ml.
RF (1 ml) = RF containing heat inactivated human serum.
RPMI (1 ml) = RPMI 1640 + 10% FCS
HIgG (1 ml) = Human IgG fraction from a serum from a patient suffering from an amoebic liver abscess, 1 mg/ml.

NNHS (1 ml) = Native normal human serum.

capacity of RIgG (130 μg/ml final concentration) to inhibit the ACA against K 562 target cells. This inhibition of ACA can be enhanced with RIgG plus RF. As a control we replaced RF containing serum by heat inactivated normal human serum. No difference between the two latter assays could be detected with regard to ACA.

Complement in form of native normal human serum (NNHS) was able to inhibit the ACA as well. After 60 minutes incubation K 562 lysis was only 16%. The addition of RIgG led to an augmented inhibition of ACA (10% K 562 lysis after 60 minutes) compared to NNHS alone. No further potentiating effect could be seen by the addition of RF (11% K 562 lysis), (data not shown in detail).

Lysis tests:

Amoebae's lysis test (AL) already occurs when amoebae are brought together with NHS as a source of complement. About 50% of amoebae are lysed by the activation of complement. Amoebae's lysis increases to 60% when incubated with NNHS plus human antibodies to amoebae.

Table 1 shows complement mediated amoebae's lysis after incubation at 37°C for 30 minutes. The addition of RIgG (25 μg/ml final concentration) to NNHS did not increase the lysis rate compared to human serum alone. Surprisingly, incubation of amoebae, NNHS, RIgG plus RF gave a marked reduction of amoebae's lysis. Control experiments with RF alone revealed AL similar to the spontaneous amoebae's lysis.

In the next set of experiments human antibodies directed against amoebic antigens (HIgG) were used in the amoebae's lysis test together with NNHS as source of complement. Again NNHS alone lysed 55% of amoebae. NHS plus HIgG (0.25 mg/ml) produced about 62% amoebae's lysis. No further increase in the rate of amoebae's lysis could be obtained by the addition of RF (**Table 2**).

DISCUSSION

The capability of pathogenic strains of **Entamoeba histolytica** to lyse various cultured target cells has been described by several authors using diverse experimental approaches[1,13,18,23,25]. Most probably structures of the amoebae's surface membrane are

involved in the cytotoxic action, presumably by mediating target and effector cell contact via a lectin like interaction[19]. Serum factors capable to inhibit the amoebae's cytotoxic activity are obviously interfering with the recognition of the target cell[13,14].

Antibodies directed against amoebic plasma membrane antigens inhibit the ACA. However, after binding to the membrane the antigen-antibody complexes are capped and partially shedded or internalized. The reappearance of a smaller number of new surface antigens are thought to be responsible for ACA inhibition. A further amplification of these effects by RF was considered, because it has been reported that RF react with IgG which has undergone severe conformational change such as antigen binding[5]. In our assay no modulation of ACA by RF could be detected. These results can also be presumed for the situation **in vivo**.

The slightly enhanced inhibition of ACA during incubation with RIgG plus HNHS could possibly be due to cytokines, such as interferon-gamma or tumor necrosis factor, which are known to exert antiparasitic activity and occur in small amounts in NHS.

Complement can lyse about 50% of amoebae after 30 or more minutes incubation at 37°C[15,20]. Beside this activation of the complement cascade via the alternative pathway, human antiamoebae antibodies also activate the classical pathway of complement[24]. A total amoebae's lysis rate of more than 60% after 30 minutes incubation at 37°C could be demonstrated in our assay. We expected RF to act as a second antibody and a complement magnet in form of a Clq binding site. In our hands RF did not show any influence on amoebae's lysis together with NNHS and HIgG. Forty per cent of amoebae are apparently resistant to complement lysis even under this maximally complement activated condition and prolonged incubation.

In presence of RIgG we could not show an activation via the classical pathway of complement in the amoebae's lysis assay. By adding RF we found a reduction of amoebae's lysis. Those findings might be due by a missing activation of the classical pathway of the human complement system by these RIgG. However RIgG may react with RF. Amoebae antigen-RIgG-RF complexes on the amoebae's surface may lead to a transient immobilisation of the membrane followed by capping phenomena, partial ingestion and partial shedding of these immune complexes which in turn activate and consume complement far away from the amoebae's surface.

Another possible mechanism for our findings is that these complexes on the amoebae's surface membrane may block C3b binding sites and hence inhibit assembling of C3 convertase of the alternative pathway of complement activation. So the complex C3BbP cannot be formed sufficiently on the amoebae's surface.

In our study we could show that RF neither has an effect on amoebae's cytotoxic activity nor on complement mediated amoebae's lysis **in vitro**. Our results suggest that the interaction between antibodies against amoebic antigen and human IgG (RF) do not exert a protective role against extraintestinal amoebiasis.

RESUMO

Interação do fator reumatóide e Entamoeba histolytica

Testes para citotoxicidade e lise amebiana foram utilizados para demonstrar uma possível interação entre o fator reumatóide e a **Entamoeba histolytica**. A atividade citotóxica amebiana foi inibida pela IgG antiameba de coelho purificada através de cromatografia. Constatou-se inibição aumentada com IgG antiameba de coelho mais fator reumatóide. A mesma inibição acentuada da atividade citotóxica amebiana pôde ser constatada quando se substituiu o fator reumatóide por soro humano normal, inativado pelo calor, como controle. Cerca de 50% de lise amebiana ocorreu quando as amebas foram misturadas com soro normal humano como fonte de complemento. A lise amebiana aumentou para 60% quando incubadas com soro humano normal, acrescido de anticorpos humanos antiameba. Nenhum aumento adicional pode ser obtido pela adição de fator reumatóide. Usando IgG antiameba de coelho em vez de anticorpos humanos, a proporção de lise não aumentou. A incubação de amebas com soro humano normal, IgG antiameba de coelho e fator reumatóide reduziu acentuadamente a lise amebiana. O fator reumatóide não teve efeito na atividade citotóxica amebiana, nem na lise amebiana mediada pelo complemento **in vitro**.

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