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A Possible Interconnection of Cholesterol Overloading and Phagocytic Activity of the Monocytes in the Prone to Rheumatoid Arthritis Individuals

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Abstract

Background: The abnormalities of lipid metabolism were demonstrated in the individuals, who later develop rheumatoid arthritis (RA). Trivial infections are known to contribute to atherosclerosis. We showed that first degree relatives of RA patients suffer from frequent and lingering trivial infections. The interconnection between the disturbance of lipid metabolism and the increased trivial infection burden is hypothesized in this group. Mononuclear phagocytes (MP) are the important players in RA pathogenesis, antiinfectious defense and atherosclerosis. The aim was to investigate the interconnection between the involvement of MPs in cholesterol metabolism, their phagocytic activity and the burden of trivial infections in the RA patients, their relatives and healthy individuals without autoimmune diseases in family history.

Methods: The following parameters were studied: intracellular cholesterol content (colorimetric), membrane cholesterol content and microviscosity (fluorescent), engulfment and digestion (radioisotope); reactive oxygen species generation (chemiluminescence).

Results: In relatives MPs and their cell membranes were overloaded with cholesterol; microviscosity of cell membranes and membrane annular lipid regions was increased, cholesterol accumulation in the cells being in strong correlation with the incidence and duration of the trivial infections. In RA group only increased microviscosity of annular lipid regions was revealed. In the patient and relative groups the delayed engulfment and the slowed down time to reach the peak of reactive oxygen species generation after MP stimulation were revealed.

Conclusion: We speculate that the abnormalities in monocyte – cholesterol interaction can exacerbate the insufficiency of antiinfectious defense and promote the aggravation of the infectious syndrome, which is known to be a risk factor of RA.

Keywords: Rheumatoid arthritis; Family study; Lipids; Monocytes; Phagocytosis

Abbreviations

CPM: Count Per Minute – the unit of index measurement in the radioisotope experiments; CE: Cholesteryl Ester; HIs: Healthy Individuals not hereditary tainted with autoimmune diseases as a control; HIy: HIs of a younger (32.3 ± 3.0 years old) subgroup; HIo: HIs of an elder (52.67 ± 2.5 years old) subgroup; HMP: High Molecular weight digestion Products; HRs: First degree female relatives of the RA patients, assigned as healthy after clinical examination; LDL: Low Density Lipoproteins; LMP: Low-Molecular weight digestion Products; OP: Object of Phagocytosis; OZ: Opsonized Zymosan; Ps: Patients with advanced rheumatoid arthritis; PEC: Pyrene Eximerization Coefficient; RA: Rheumatoid Arthritis; ROS: Reactive Oxygen Species.

Background

The early development of atherosclerosis complicates rheumatoid arthritis (RA), being considered to be due to the chronic inflammation [1,2]. At the same time, blood donors who later develop RA have a proatherogenic lipid profile 10 years before onset of the disease [3].

We showed that the first degree relatives of RA patients suffer from frequent and lingering trivial infections [4]. Our further observations revealed that the infection incidence and duration come down to norm in 3 years after the disease onset [5]. We assume any defects in anti-infectious immunity in the predisposed to RA individuals, the following links being the week ones – phagocyte dysfunction, low levels of some innate immunity factors, antibody production abnormality. The imbalance might be in a certain degree convertible, so in 3 years after the RA onset some compensatory mechanisms start up, that being enough for getting rid of the clinical manifestations of the infections but not enough for the full control of the bacterial colonization. The latent insufficiency of the antiinfectious defense may become evident under the influence of various factors and is due to the infectious complications of RA.

It is known that activation of the acute phase response, via inflammation or infection alters the lipid profile and can provoke atherosclerosis [6]. In particular, the opsonizing of the native low density lipoproteins (LDL) by the acute phase factor C-reactive protein (CRP) leads to the uptake of the CRP/LDL by macrophages by macropinocytosis, this process being due to foam cell formation in

human atherogenesis [7]. So the burden of several common viral and bacterial infections may contribute to atherosclerosis and risk of clinical vascular events [8].

On the other hand lipids are the well-known modulators of the various cell functions, phagocytosis in particular [9,10].

We suggested that there might be the interconnection between the disturbance of lipid metabolism and the increased trivial infection burden in the individuals, predisposed to RA.

For the research, we chose the mononuclear phagocytes, as these cells are the important players in the pathogenesis of RA, anti-infectious defense and atherosclerosis.

Materials and Methods

Groups

Twenty four women (Ps, 52.76 ± 15.0 years old) with advanced RA (duration – 14.88 ± 10.6 years). Fifteen Ps (62.5%) were seropositive for rheumatoid factor, 16 (66.7%) for autoantibodies to cyclic citrullinated peptides, disease activity was 3.18±1.7 au (mean ± standard error) of DAS28-ESR, HAQ – 2.03 ± 1.02 au. Nineteen Ps received methotrexate, 5 – sulfasalazine.

Twenty four first degree female relatives of these patients, assigned as healthy after clinical examination and routine paraclinical check-up for a set of inflammatory and autoimmune diseases (HRs, 35.3 ± 0.5 years old). This group included 16 daughters, 7 sisters of the RA Ps and 1 woman whose mother and sister suffer from RA. For the fragment of the research project presented in this article, we formed a group of HRs, 14 of which (58%) were the carriers of shared epitope alleles in genome. All of them were seronegative for rheumatoid factor and antibodies to cyclic citrullinated peptides. They showed no symptoms or MRI signs of arthritis (MRI of the hand joints was performed with the consent of these persons and with the permission of the ethical committee, which was based on the conclusion of two qualified experts in this field); as well as no stably increased ESR or serum C-RP levels (except the infectious periods) were revealed in this group.

Twenty four individuals had no chronic disease and gave a statement of no autoimmune disease family history (HIs). They underwent the clinical examination. None of diagnostic laboratory RA markers or the laboratory signs of inflammation (ESR, C-RP) were revealed in this group.

The data eliciting cell – lipid interaction were analyzed in the comparable by age groups, the corresponding indexes in the HIs are represented in the younger (HIy, 32.3 ± 3.0 years old) and the elder (HIo, 52.67 ± 2.5 years old) subgroups.

The exclusion criterion in all the groups was the presence of known risk factors of infections as smoking, habitual alcoholism, diabetes mellitus, and concomitant chronic diseases [11] (Table 1).

Study Design

Anamnestic information on infections was collected by a physician qualified in Russia as a specialist in rheumatology at semiannual in-hospital 2-day-visits for the most of the HR and Ps, for the HI infection information was from the one year preceding enrolment. The study persons were asked for symptoms suggesting infections, experienced during the preceding every 6 - 12 months. The information from out clinic documents was sought whenever a general practitioner had been visited. Only those episodes judged by the rheumatologist to truly indicate an infection were scored (there was no formal list of criteria to be addressed during this procedure). In the case of exacerbation of a chronic infection, the diagnosis was established by a specialist in the corresponding medical area. The following parameters of the infectious syndrome were analyzed – the incidence of the infectious episodes and the overall duration of these episodes per year. If the clinical examination was carried out every 6 months, the number and duration of infection for the two halves of the year were summarized.

Groups	Age, years
RA* patients, n=24	52.76 ± 15.0
I degree healthy female relatives, n=24	35.3 ± 0.5
Healthy women without RA in family history (control), n=24	42.6 ± 0.9
of which*:	
"Young" controls, n=12	32.3 ± 3.0
"Old" controls n=12	52.7 ± 2.5
*Given the fact that the age difference between the patient RA and their relative groups was significant, while the studied lipid indices are known to depend on the age of the subject, in order to analyze indices in comparable by age groups, control women were separated into the "young" and "old" subgroups	

Table 1: Sample sizes of the study groups.

The study was approved by the Ethical Committee of the Kazan State Medical Academy, Kazan, Russia (Permit nr 1/2002). The consents from all the patients involved in the study, including consents to participate in the study and consents to publish the results were received.

Objects

Peripheral blood monocytes were taken in the periods without any clinical symptoms of an infection (all the groups) and any routine laboratory signs of inflammation (HRs and HIs).

Monocytes were isolated on Ficoll – Urografin density gradient. Cell membranes were isolated using a universally accepted method, the membrane enriched fraction being identified by the presence of 5'nucleotidase [12]. Intracellular cholesterol content (10 x 7 cells) was assessed by the enzymatic colorimetric analysis after ultrasonic disintegration (Vital, Russia).

Membrane cholesterol content was estimated using filipin (Sigma, USA), forming fluorescent complexes with the membrane cholesterol (λem=500-510 nm, λex=358 nm). Monocyte (10 x 6) suspensions were incubated in the 10, 20, 30, 40, 50, 60, 70 mkM filipin solutions. The measurements were carried out on the fluorimeter MPF - 44B (Perkin Elmer, USA) in the quartz cuvettes.

Cell membrane microviscosity was assessed using pyrene (Sigma, USA). Pyrene solution in ethyl alcohol was added to the cell suspensions (10 x 6) to the final concentrations of 10, 15, 30, 60 mkM at constant stirring. Pyrene eximerization coefficient (PEC) was estimated as a ratio of the fluorescence intensity 460 nm (eximers)/370 nm (monomers), λex=335 nm. Annular lipid regions microviscosity was estimated in the membrane suspensions, incubated in the 15 mkM

pyrene solution as PEC, pyrene fluorescence being excited by the inductive - resonant energy transfer from tryptophan of the membrane proteins (λex=285 nm). The intensity of tryptophan fluorescence quenching by pyrene was measured as the difference of tryptophan fluorescence intensity before and after pyrene invasion (a percentage from the tryptophan fluorescence intensity before the probe invasion).

Engulfment and digestion were estimated within 24 hours using a radioisotope method [13]. The object of phagocytosis (OP) - Staphylococcus aureus Wood strain was grown in the media with 14°C – labelled amino acids and opsonized with pooled native human serum (group IV). The OP suspension was added to cell suspensions (103 microbial units/cell) with the following incubation at 37°C for 30 minutes, and then the unbound material was removed by centrifugation. Then the cells were washed twice. The first measurements were done immediately following the 30 minute incubation, the next - in 2, 4, and 24 hours incubation at 37°C. The cells incubated for further 2, 4 and 24 hours were centrifuged and washed twice to remove all accumulated products of digestion from suspensions.

Thus, each tested sample contained only those digestion products that had accumulated within a certain time period. The intensity of digestion of bacterial proteins to low-molecular weight peptides (LMP) during first 30 minutes of incubation was assessed as a value difference between the radioactivity of cell suspension supernatants and bacteria suspension supernatants after treatment of them with 20% trichloroacetic acid. As the supernatants received following the first 30 minutes of incubation with labeled bacteria contained OP both unbound to the cells and already degraded ones, the level of the high molecular weight products (HMP) was not taken into consideration, since it was not possible to determine the portion of OP, unbound to the cells.

After cell sedimentation the following samples were measured (Beta analyzer POMA, Ukraine): (a) total cell-bound radioactivity of the label; (b) intracellular label radioactivity (following the removal of surface membrane proteins by trypsin treatment); (c) radioactivity of supernatants (HML- and LMP-bound label); (d) LMP-bound label radioactivity (after HMP precipitation by 20% trichloroacetic acid). The measured values were expressed in CPM – count per minute. Under the selected experimental conditions the cell counts were not essentially decreased in the samples, and their viability estimated by trypan blue staining was ~ 96%.

Reactive oxygen species (ROS) generation was estimated by luminol-dependent chemiluminescence technique (chemiluminometer designed by Santalov BF, Pushchino, Russia). Real time registration was performed every 4 sec in the thermostated plastic chambers with continuous mixing of cell suspensions (sample volume 0.2 ml, cell density – 10 x 6/ml, and concentration of OZ 0.25 mg/ml). The following parameters were measured: spontaneous level of ROS production (arbitrary units, au), total ROS production estimated as an area under the curve of time – chemiluminescence intensity dependence within 40 minutes after opsonized zymosan (OZ, Sigma, USA) addition (arbitrary units, au), and time of occurrence of peak ROS production (min).

Statistical analysis

Mann - Whitney criterion, Student T-criterion for the independent samples, regression analysis. For statistical analysis, the results of all the samples were tested for normality of the distribution using the Pearson criterion.

Results

There were some slight proatherogenic shifts in the serum lipid levels in the HRs and Ps (data are not shown). In the HR monocytes cholesterol content was 1.9 times higher than that in the comparable by age control HIy group) this index in the Ps being close to that in the HIos (Figure 1).

Only in the HRs regression analysis revealed a direct and strong relation between the intracellular cholesterol and the infection syndrome parameters (5.6 ± 0.6 infectious episodes per year with overall duration 46.1 ± 7.7 days per year) – RI=0.83, $p<0.002$. In the HIs (3.2 ± 0.4 episodes with the duration 23.2 ± 3.3 days) and Ps (2.9 ± 0.5 episodes with the duration 22.0 ± 5.6 days) there was no relationship between the intracellular cholesterol and the infection syndrome parameters.

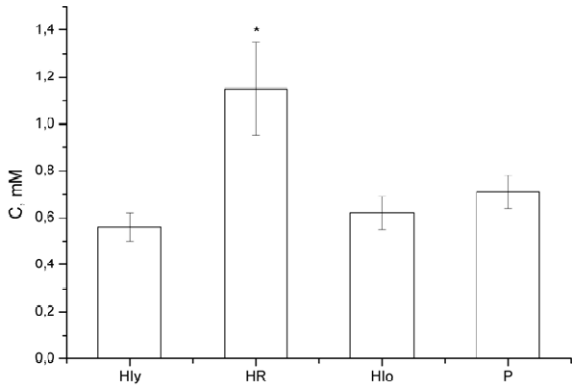

Figure 1: Cholesterol content in the monocytes of the RA patients (P, n = 10), their relatives (HR, n=10), and the healthy individuals of the young (HIy, n=12) and old (HIo, n=8)) age groups, mean ± standard error, *$p<0.05$ when compared with the HIy group (Student T-criterion for the independent samples).

Fluorescence of the cholesterol – filipin complexes in the cell membranes of the HRs was reliably more intensive than that in the HIy group (Figure 2a). In the Ps the corresponding indexes were close to that in the HIo group in all the points of the concentration curve (Figure 2b). While incubation of the HR and P monocytes in the 50, 60, 70 mkM filipin solutions, the fluorescence of cholesterol – filipin complexes dropped then sharply increased (Figure 2) that might be due to the membrane solubilization owing to the overloading with probe molecules. This effect can testify the peculiarities of the membrane phospholipid composition or the decreased phospholipid/cholesterol ratio.

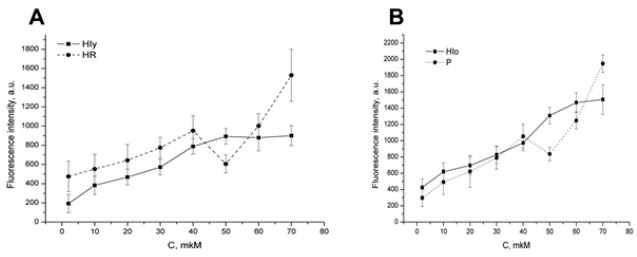

Figure 2: The dependence of fluorescence of cholesterol – filipin complexes in monocyte cell membranes on filipin concentration in the RA patients (P, n=10), their relatives (HR, n=10), and healthy individuals of the young (HIy, n=8) and old (HIo, n=8) age control groups, mean ± standard error.

The difference is reliable ($p<0.05$) when compared the indexes of the HR and HIy groups in all the points of the concentration curve (Student T-criterion for the independent samples). The differences between the indexes of the adjacent points of the concentration curves (50, 60, 70 mkM filipin solutions) in the HRs as well as in Ps are reliable ($p<0.05$, Student T-criterion for the dependent samples).

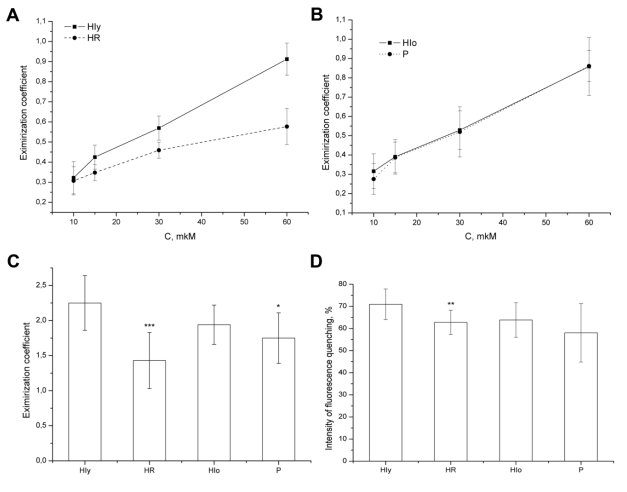

Figure 3: Pyrene eximerization coefficients (au) in the cell membranes (3A,3B), annular lipid regions (3C) and intensity of tryptophan fluorescence quenching (%) in the presence of pyrene in annular lipid regions (3D) of monocytes in the RA patients (P, n=10), their relatives (HR, n=10) and the healthy individuals of the young (HIy, n = 10) and old (HIo, n = 10) age control groups, mean ± standard error.

In the HRs PECs were found to be reliably decreased in all the points of the concentration curve (Figure 3a). When pyrene fluorescence was excited by the inductive - resonant energy transfer from tryptophan of the membrane proteins, the PEC was found to be decreased as well (Figure 3c). So, the membrane lipid bilayers and annular lipid regions of membrane proteins were more viscid in this group. Besides, the intensity of tryptophan fluorescence quenching in the presence of pyrene in annular lipid regions of monocyte membrane proteins was reliably decreased (Figure 3d).

In the Ps the PECs absolutely coincided with those in the control group (Figure 3b). The intensity of tryptophan fluorescence quenching was also close to the control index (Figure 3d). However the micro viscosity of the annular lipid regions was reliably increased (Figure 3c).

In Figure 3A, the HRs the PEGs is reliably increased at the pyrene concentrations 20-60 mM ($p<0.05$, Student T-criterion for the independent samples). Figures 3C and 3D the reliable difference ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$) when compared to the indexes in the corresponding age group of HIs ($p<0.05$, Student T-criterion for the independent samples). The difference is reliable ($p<0.05$) when compared the indexes of the HR and HIy groups in all the points of the concentration curve (Student T-criterion for the independent samples). The differences between the indexes of the adjacent points of the concentration curves (50, 60, 70 mkM filipin solutions) in the HRs as well as in Ps are reliable ($p<0.05$, Student T-criterion for the dependent samples). The difference is reliable ($p<0.05$) when compared the indexes of the HR and HIy groups in all the points of the concentration curve (Student T-criterion for the independent samples). The differences between the indexes of the adjacent points of the concentration curves (50, 60, 70 mkM filipin solutions) in the HRs as well as in Ps are reliable ($p<0.05$, Student T-criterion for the dependent samples).

The difference is reliable ($p<0.05$) when compared the indexes of the HR and HIy groups in all the points of the concentration curve (Student T-criterion for the independent samples).

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Spontaneous ROS production by the P monocytes was reliably increased (Figure 4a) while in the HRs only a tendency was revealed ($p=0.055$). Total OZ-stimulated ROS production was reliably enhanced both in the Ps and HRs (Figure 4b). The time to reach the peak of OZ-stimulated ROS generation in the Ps and HRs was in two times longer than that in the HIs (Figure 4c).

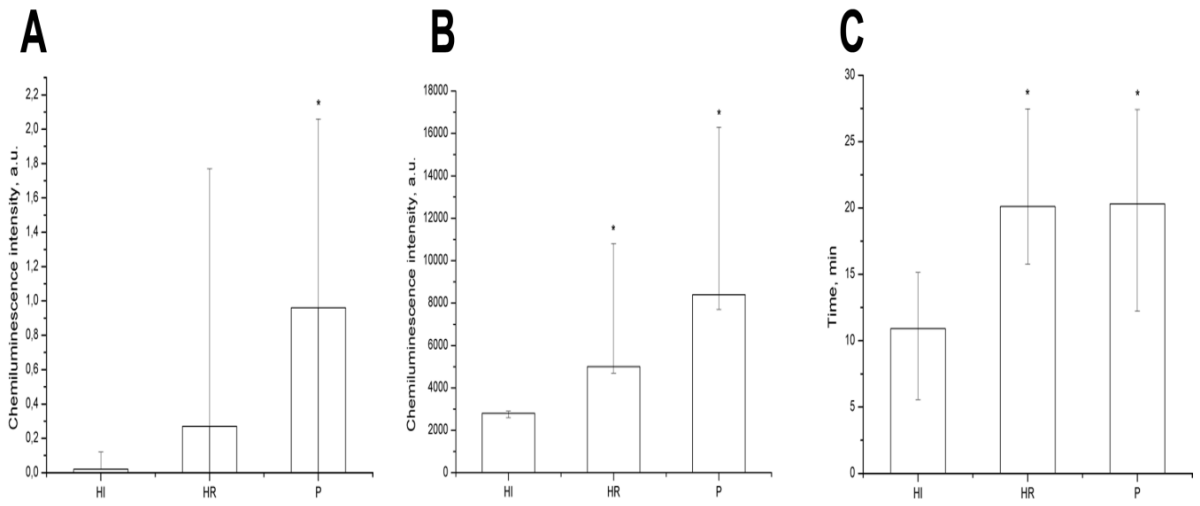

Figure 4: Spontaneous ROS generation (4a), total zymosan-stimulated ROS generation (4b) and the time to reach the peak of zymosan-stimulated ROS generation by the monocytes of the RA patients (P, n=10), their relatives (HR, n=7) and healthy individuals (HI, n=7), median ± 5, 95 percentile The indexes are reliable (p<0,05) when compared to that in the HIs (Mann-Whitney test).

There was a pronounced difference in the dynamics of OP engulfment in the groups (Figure 5a). In the HIs the amount of the engulfed C14- OP was almost at the peak in the first 30 minutes of the experiment, while in the Ps the ingestion was maximal at the interval of 2 - 4 hours from the beginning of the process, the amount of material engulfed by the HI cells in this time interval was decreasing. In the HRs the process of engulfment of the labelled bacterial particles was slowed down as well, but it was also distinctly decreased. The overall 24 - hour digestive activity of the HR cells was reliably lower as compared to that in the HIs (Figure 5b). The highest digestive activity was observed in the Ps.

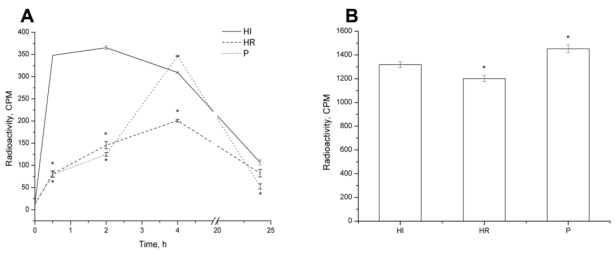

Figure 5: Radioactivity of the intracellular label (5a) and the overall 24 - hour digestive activity of the monocytes of the RA patients (P, n=24), their relatives (HR, n=24), and healthy individuals (HI, n=24), not hereditary tainted with autoimmune diseases, mean ± standard error. *p<0.05 when compared with the HIs (Student T-criterion for the independent samples).

Discussion

It can be hypothesized that the excess cholesterol content in the HR monocytes is due to the uptake of modified low density lipoproteins (LDL).

Firstly, in the HRs we have revealed the increased serum levels of lipid peroxidation products and oxidized proteins (unpublished data), though the mechanism of oxidative stress in this group is not yet fully clear. So, the supposition about the abundant formation of oxidized LDL in the bloodstream of the predisposed to RA individuals is rather acceptable. Secondly, just uptake of the modified LDL via scavenger receptors is not limited on the feedback as opposed to the native LDL taken via highly regulated special receptors [14-16]. Getting rid of the excess cellular cholesterol occurs via cell membranes [17,18]. Cholesterol may become highly concentrated in the cell membranes, violating the membrane functional properties, in particular – membrane fluidity and signal transduction from membrane-coupled receptors, these processes being a hallmark of early atherogenesis [19-21].

So, in the HRs the overloading of monocytes and their membranes with cholesterol, the abnormal microviscosity of the membranes and particularly of annular lipid regions of the membrane proteins may be directly due to the abnormal functioning of the cells, namely - slowed down and decreased engulfment, lowered digestive activity and the lengthened time needed to reach the peak of ROS generation.

The other abnormalities of monocyte membranes revealed in the HR group – the effect of the membrane solubilization in the experiments with filipin and the decreased intensity of tryptophan fluorescence quenching in annular lipid regions – may be due to the peculiarities in the phospholipid composition or the reproportioning of cholesterol/phospholipids. Cholesterol accumulation in the cells leads to the translocation of cytosolic membranes and the following release of fatty acids from membrane phospholipids required for the

cholesterol etherification [22]. These processes may explain the indicated above abnormalities.

We have shown that the HRs suffer from frequent and prolonged trivial infections and the regression analysis revealed the connection of the intracellular cholesterol content with the repeated infectious episodes in this group. Really the so-called "infectious burden" – common bacterial and viral infections – has been hypothesized to contribute to the development of atherosclerosis [8,23]. The probable conditionality of the abnormal mononuclear phagocyte functioning by the cell cholesterol overloading elucidated another aspect of the problem. We speculate that the abnormalities in monocyte – cholesterol interaction can exacerbate the insufficiency of antiinfectious defense and promote the aggravation of the infectious syndrome, which by-turn is known to be a risk factor of RA.

The results of the experiments in the Ps look to be in contradiction with the increased mortality from cardiovascular complications of atherosclerosis in RA. In connection with this we would like to observe the following features of atherosclerosis in the RA patients. The majority of the authors revealed that lipid profiles in RA are different from those observed in the general population at risk of cardiovascular disease. In the Ps with active RA an increased risk of cardiovascular disease is associated with the lower total cholesterol and LDH levels [24,25]. At that all the authors speculate that the abnormalities of lipid metabolism in the RA patients are due to the chronic inflammatory process and oxidative stress but not correlate with hereditary factors as it is usual in the general population [26].

Atherosclerotic plaques of RA patients differ morphologically from those observed in the general population, with less histological evidence of atherosclerosis but far greater evidence of inflammation and instability (with monocyte-derived macrophages in the title role) [27,28]. A relatively smaller contribution of LDL to the atherosclerosis development in RA might be not a single reason for the lack of differences in the indexes between the P and HI groups.

Oxidative stress plays an important role in the pathogenesis of RA and the increased levels of oxidized LDL were demonstrated in plasma and synovial fluid of RA patients [29,30]. These findings together with the impaired cholesterol efflux capacity of HDL in the RA patients allow expecting the overloading of P monocytes and their membranes with cholesterol [31].

The differences in the HR and P monocytes state may be due to the differences in the extent of proatherogenic shifts – the initial in the HRs and the advanced in the late RA. It well known that the oxidized LDL infiltrates arterial walls, provoking the migration of monocytes to the intima [32,33]. Once resident in the arterial intima, monocytes accumulate lipids, transforming into the foam cells which should undergo apoptosis [27]. The ratio penetrating monocytes/foam cells decreases until a one-to-one ratio is achieved in the late plaques [34]. The other fate of the lipid – laden cells is to migrate back into the bloodstream, this being the mechanism of removing the lipids and vessel wall clearance. We guess that the revealed in the HRs cholesterol overloaded cells with the rigid membrane, are just the same ones, leaving arterial intima, whereas in the late RA the most of the monocyte - derived foam cell undergo apoptosis in the lipid infiltrated intima.

The more pronounced oxidative stress in the P group might be due to the increased ratio of the oxidized cholesterol in the monocytes and their membranes. There is the evidence of the intracellular cholesterol oxidation and oxysterol droplets in macrophages [35]. Used in our experiments colorimetric cholesterol estimation is based on the lipid oxidation by the bacterial cholesteroloxidase with hydrogen peroxide formation, the later reacts with phenol and aminoantipyrine with the colored product formation. So this method fails to reveal the previously oxidized cholesterol which could constitute a greater share of the intracellular lipid inclusions in the Ps. The detection of the membrane cholesterol might face the same problem. Filipin – cholesterol interaction in the membrane is known to be spatial and the stereo-specific binding of cholesterol to sterol-sensing domains is not duplicated by oxysterols [36,37]. So, the significant ratio of the membrane cholesterol might be oxysterol in the RA group, while we revealed only native molecules. Cholesterol auto-oxidation product – 25-hydroxycholesterol – changes the position, orientation, and solvent accessibility of cholesterol and can trigger cholesterol trafficking away from the plasma membrane [38]. And though the various auto-oxidation products inserted into the membrane lipid bilayers demonstrate the cholesterol – like effect on the membrane functions, it is less efficient, and some of these products show no effect [39].

In the P group we revealed the effect of the membrane solubilisation by filipin testifying to the peculiarities in the membrane phospholipid composition or the reproportioning of phospholipids/cholesterol which by-turn might be probably due to the constant membrane lipid peroxidation in the conditions of the increased ROS generation. Micro viscosity in the annular lipid regions of monocyte membrane proteins in this group was increased that being one of the causes of the delayed engulfment and the slowed down time to reach the peak of ROS generation. We speculate that the abnormalities in monocyte – cholesterol interaction can exacerbate the insufficiency of antiinfectious defense and promote the aggravation of the infectious syndrome, which is known to be a risk factor of RA.

Conclusion

In the HRs cholesterol overloading might lead to the functional abnormalities of the mononuclear phagocytes, and to the deepening of the antiinfectious defense insufficiency. The increased microviscosity of annular lipid regions of membrane proteins might be partly due to the delayed engulfment and the slowed down time to reach the peak of ROS generation in the HRs and Ps. We speculate that the abnormalities in monocyte – cholesterol interaction can exacerbate the insufficiency of antiinfectious defense and promote the aggravation of the infectious syndrome, which is known to be a risk factor of RA.

Authors' contributions

MA conceived and designed the study, designed and executed all the experiments, supplied samples, clinical and laboratory data analyzed and interpreted all the results and wrote the manuscript. AZ designed and executed experiments with the use of the fluorescent methods and interpreted their results. AG executed experiments, detecting cholesterol intracellular content and analyzed their results. YF executed chemiluminescent investigations and analyzed their results. AT conceived and designed the study. All authors edited the manuscript, read and approved the final version of the manuscript.

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