Increased propensity for amyloidogenesis in male mice

S. Shtrasburg[1,5], M. Pras[1,5], M. Dulitzky[2,5], C. Pariente[2], R. Gal[3,5], A. Mor[4,5], A. Livneh[1,5]

[1]Heller Institute of Medical Research and [2]Department of Obstetrics and Gynecology, Sheba Medical Center, Tel-Hashomer; [3]Department of Pathology, Rabin Medical Center, Golda Campus, Hasharon Hospital, Petach-Tikva; [4]Department of Medicine, Meir General Hospital, Kfar Saba; and [5]Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel.

Abstract

Background

The male sex is a risk factor for reactive amyloidogenesis in several disease entities. Environmental, socioeconomic or genetic factors may underlie this male preponderance. This study was aimed at discovering whether male sex predisposes to reactive amyloidosis also in mice and to elucidate some of the hormonal associations of this risk.

Methods

Male and female Swiss mice were subjected to an established amyloid induction protocol and the amount of their splenic amyloid was determined and compared. The effect of estrogen, progesterone, testosterone and adrenalin on amyloidogenesis was studied in both sexes by administering these hormones during amyloid induction and comparing the amount of splenic amyloid of the study mice with the control mice which received the amyloid induction protocol alone.

Results

Amyloid deposition appeared to be more abundant in male mice. This gender difference was not associated with any of the 3 sex hormones tested. Despite an expected increment, adrenalin caused an attenuation of amyloid deposition.

Conclusions

The preferential expression of reactive amyloidosis in male mice seems to be unrelated to the common sex hormones. Increased production of other hormones such as adrenalin, or perhaps an augmented susceptibility to their effect, may cause gender differences by suppressing female amyloidogenesis. Our study favors the hypothesis of genetic predisposition as the mechanism leading to sex differences in amyloidogenesis. Further validation of our findings in gonadal ablated models and other amyloid induction protocols is warranted.

Key words

Amyloid A, amyloid enhancing factor, animal model, adrenalin, sex hormones.

Shmuel Shtrasburg, BSc; Mordechai Pras, MD; Mordechai Dulitzky, MD; Clara Pariente, MSc; Rivka Gal, MD; Adam Mor, MD; and Avi Livneh, MD.

Please address correspondence to: Shmuel Shtrasburg, BSc, Heller Institute of Medical Research, Sheba Medical Center, Tel Hashomer 52621, Israel. E-mail: shmuels@sheba.health.gov.il

Received on December 1, 2003; accepted in revised form on March 31, 2004.

Introduction

Reactive amyloidosis is a condition complicating a variety of infectious, inflammatory, malignant and genetic diseases (1-4). It is defined by the deposition of amyloid A (AA) in tissues and is characterized by elevated serum levels of SAA among the other acute phase proteins (5-8).

Three lines of evidence suggest that individuals of the male sex harbor an increased risk for the development of amyloidosis. First is our experience with familial Mediterranean fever (FMF), a periodic febrile disease strongly associated with AA amyloidosis in untreated individuals. In our FMF cohort amyloidosis was more common in men (9). A similar observation has been made by others (10). Secondly, male predominance in reactive amyloidosis has been observed in several clinical entities in addition to FMF, including inflammatory bowel disease (11) and psoriasis (12). Third, in juvenile chronic arthritis (JCA), although much more common in women, the number of men and women with reactive amyloidosis is equal (13), suggesting a male preponderance in amyloidogenesis in this entity as well.

The cause of the preferential expression of reactive amyloidosis in males is unknown. Environmental and socioeconomic, as well as genetic factors, may all play roles in this finding. A controlled trial using an animal model of amyloidogenesis may shed light on this enigma.

Indeed, concomitantly with our clinical observations in men, we also noted (but never studied systematically) in mouse models that female mice as compared to males are less prone to develop amyloidosis. The difference was manifested by lower quantities of amyloid in the spleen and a larger number of individual mice that remain amyloid-free. This is consistent with another recent noteworthy observation of suppression of amyloidogenesis during pregnancy (14).

The present study was therefore undertaken to find out whether male mice are indeed at a higher risk to develop amyloidosis as compared to female mice and to explore the factors that may underlie such a trend.

Methods

Experimental animals

Male and female Swiss mice, 8 to 18 weeks old, originating from Survey's Veterinary Institute, Beth Dagan, Israel, were used in the current set of experiments. The study was performed in accordance with the guidelines of the animal experimentation committee of our hospital.

Induction of amyloidosis by casein and quantification of amyloid deposition

Amyloidosis may be induced experimentally in mice by eliciting chronic inflammation using two possible approaches: (a) the classical method, in which amyloidosis is induced within 2-3 weeks (7, 15); and (b) a faster method in which amyloidogenesis is shortened to a few days. In the present study, classical amyloidosis was induced by casein according to established protocols (15, 16). Briefly, the mice were injected subcutaneously (s.c.) with 0.5 ml/day of 15% vitamin-free casein suspended in 0.02N NaOH for 5 days/week for 3 weeks. Following this treatment the animals were killed and the presence and amount of amyloid in their spleens were studied using the crush-and-smear (C & S) technique and a 5-grade semi-quantitative scale (16-19).

Enhanced induction of amyloidosis

The production of amyloid enhancing factor (AEF) and the induction of enhanced amyloidogenesis were performed as described previously (16,18, 19). Briefly, AEF was prepared from acetone-treated homogenates of spleens of pre-amyloidotic mice and injected intravenously into the studied mice (1 μg/animal). $AgNO_3$, an inflammation-inducing agent, was administered concurrently to the AEF-primed mice over 3 successive days (2%, 0.5 ml/day, s.c.) (14,16,18-22). Six days after the first $AgNO_3$ injection, the mice were killed and their spleens were examined for the presence and amount of amyloid deposition using the C&S technique. Experiments of shorter duration, lasting 2 to 4 days, which were performed to study the effect of testosterone (to be described later) before ample amyloid deposi-

tion could interfere with such evaluation, were carried out as well (indicated in the text and tables). Similarly, experiments of shorter and longer duration, in addition to the standard induction protocol, were used to learn the kinetics of amyloid deposition.

Administration of female sex hormones

The possible effect of estrogen and progesterone on amyloidogenesis was studied as described (14). Briefly, 0.5 mg/day water soluble 17β-estradiol, or 1 mg/day water soluble progesterone, or the combined hormones, were injected intraperitoneally into male mice beginning on day 0 of the amyloid induction protocol until the mice were killed. Control mice received the same induction protocol but with the solvent (cyclodextrin) alone (without sex hormones) (14).

At the termination of these experiments the amount of splenic amyloid was studied as described above. In addition, serum sex hormone levels were determined in several experiments using the immunochemiluminiscent method or a radioimmunoassay (RIA), as published (14); these consistently showed a 10-20 fold rise in the hormone-treated animals as compared to the controls (14).

Administration of testosterone

Testosterone enatate (Testoviron Depot, Schering AG, Berlin, Germany) was injected intramuscularly (i.m.), 2 mg in 0.2 ml castor oil (recini oil) into female mice at the initiation of the enhanced protocol, and again after 48h (concurrently with the third $AgNO_3$ injection). Control animals received the same regimen but with castor oil only (without testosterone). At the termination of this set of experiments, the amount of splenic amyloid was determined as described above. In addition, serum testosterone levels were measured by a solid-phase RIA kit (Coat-A-Count Testosterone, DPC, Los Angeles, CA), with a sensitivity of 0.1 ng/ml and intra- and inter-assay coefficients of variation of 4% and 10%, respectively. The level of serum testosterone in the study animals was found to be 10-20 times higher than in the control mice (8-14 mu/ml vs < 1).

Administration of adrenalin

Adrenalin (Teva Pharmaceutical Industries Ltd., Petah Tikva, Israel) was injected i.p. at a dose of 4 μg/0.2 ml saline into female mice. In the casein induction protocol, adrenalin was given twice a day, 5 days per week over a period of 3 weeks, while in the enhanced protocol adrenalin was administered as above, but only over 3-4 days, according to the duration of the experiment as indicated in the results. Control groups received the same regimen but with saline alone, without adrenalin. At the termination of this set of experiments, the amount of splenic amyloid was determined as described above.

Statistical analysis

Statistical analysis was performed using the Student t-test.

Results

The amount of splenic amyloid was studied in 3 different sets of experiments, performed more than one month apart, using the same batch of AEF. In female mice, the amount of amyloid was significantly less than in male mice. The difference between the sexes was significant in 2 out of the 3 experiments, and remained significant in the combined results (Table I).

Table II compares the kinetics of amyloid deposition in male and female mice by evaluating the amount of splenic amyloid on days 4, 5, 6 and 10 after initiation of the enhanced protocols. The findings show that at day 6, the day studied in the Table I experiments, the female and male mice were already at the peak of amyloid deposition. Thus, the study suggests that the reduced amyloid amount in female mice could not be attributed to a possible delay in amyloid deposition.

While it has already been established that estrogen and progesterone have no amyloid inhibitory effects in female mice (14), their impact on male amyloidogenesis was not studied. This is of particular concern, as the possible inhibitory influence of female sex hormones in the experimental model used (female mice) could have been obscured by the endogenous hormones. However, as seen in Table III, even in male mice amyloid deposition was not reduced following exposure to the exogenous female sex steroids.

While gender differences in mouse amyloidogenesis are probably unrelated to female sex steroids, the role of testosterone has never been studied before. Table IV shows that testosterone does not promote amyloid production in the enhanced model, even though shorter duration (more sensitive) protocols were applied to allow small increments to become evident (see Methods).

Finally, as male mice more readily fight each other in captivity cages, it was speculated that the higher amyloidogenic susceptibility in male mice might be due to increased secretion of adrenergic hormones. However, as seen in Table V, adrenalin given to female mice was found to have more of an inhibito-

Table I. Amyloid deposition is reduced in female mice*.

Experiment	Gender	Positive mice of total	Mean amount of amyloid (±SD)	Pvalues**
1	Male	20/20	3.87 ± 0.39	
	Female	15/16	2.75 ± 1.00	0.00005
2	Male	6/6	3.93 ± 0.22	
	Female	7/7	3.57 ± 0.31	0.035
3	Male	9/9	4.06 ± 0.21	
	Female	12/12	3.80 ± 0.33	N.S.
Total	Male	35/35	3.93 ± 0.33	
	Female	34/35	3.27 ± 0.86	0.000075

*The experiments were performed similarly, at different points in time. All experiments were performed using the enhanced amyloid induction protocol. The duration of all experiments was 6 days.
** Pvalues were computed using the Student *t* test.

Table II. Amyloid deposition is already at a plateau level on day 6*.

Experiment no.	Gender	Duration of experiment (days)	Positive mice of total	Mean amount of amyloid (± SD)	Pvalues**
1	Male	4	9/9	3.57 ± 0.41	
	Female	4	10/10	3.68 ± 0.43	N.S.
2	Male	5	8/8	3.76 ± 0.32	
	Female	5	9/9	3.69 ± 0.31	N.S.
3	Male	6	6/6	3.93 ± 0.22	
	Female	6	7/7	3.57 ± 0.31	0.035
4	Male	10	9/9	3.96 ± 0.29	
	Female	10	9/9	3.56 ± 0.44	0.045

* All experiments were performed using the enhanced amyloid induction protocol.
** Pvalues were computed using the Student t-test.

Table III. Female sex hormones do not suppress amyloidogenesis in male mice*.

Type of experiment	Material injected	Daily amount** (intraperitoneal)	Positive mice mice of total	Mean amount of amyloid †† (± SD)
Study 1	Estradiol (water soluble)	0.5 mg	8/8	3.54 ± 0.44
Study 2	Progesterone (water soluble)	1 mg	8/8	3.74 ± 0.39
Study 3	Estradiol + Progesterone	0.5 mg + 1 mg	8/8	3.80 ± 0.44
Control †	Cyclodextrin alone	22mg	8/8	3.80 ± 0.21

* All experiments were performed using the enhanced amyloid induction protocol with a 6-day duration.
** The amounts of hormones represent the pure hormone content. The amount of the cyclodextrin is similar to the content of cyclodextrin in Study 1 to 3 experiments.
† Control mice received the enhanced amyloid induction protocol with the diluent (cyclodextrin) alone (without sex hormone).
†† The differences between the study and control mice were not significant (by the Student t-test).

Table IV. Exogenous testosterone does not promote amyloidogenesis in female mice*

Type of experiment	Duration of experiment (days)	Material injected	Amount injected** (intramuscular)	Positive mice of total	Mean amount of amyloid**** (± SD)
Study 1	4	Testosterone	2 mg	7/7	3.84 ± 0.44
Control 1***	4	Castor oil	0.2 ml	7/7	3.74 ± 0.45
Study 2	3	Testosterone	2 mg	6/6	3.62 ± 0.37
Control 2	3	Castor oil	0.2 ml	6/6	3.42 ± 0.28
Study 3	2	Testosterone	2 mg	7/12	0.45 ± 0.55
Control 3	2	Castor oil	0.2 ml	6/12	0.50 ± 0.86

* All experiments were performed using the enhanced amyloid induction protocol.
** Testosterone in castor oil (study mice) or castor oil alone (control mice) was administered in the amount indicated at day 0, and again after 48 hrs.
***Control mice received the enhanced amyloid induction protocol with the diluent (castor oil) alone (without testosterone), in the same volume as in the study mice.
**** The differences between the study and control mice from each group were not significant (by the Student t-test).

ry than a stimulatory effect on amyloidogenesis in the longer (casein) induction protocol. An inhibition trend was also observed in the 3-day shortened (AEF) protocol which, as noted in the Methods section, was carried out to increase the sensitivity of this technique.

Discussion

In this set of experiments, aimed at the elucidation of the existence and causes of gender differences in murine amyloidogenesis, it was found that amyloid deposition is more abundant in male mice as compared to female mice. This gender difference was not associated with either a retarded amyloid deposition in female mice, or with an inhibitory effect of female sex hormones, or an aggravating effect of testosterone. Adrenalin, however, which was thought to be associated with increased amyloidogenesis in male mice, was unexpectedly found to attenuate amyloid deposition.

As has already been discussed by us recently, female sex hormones may either provoke or attenuate inflammation by several mechanisms (14). Yet, if administered to female mice, female sex hormones do not affect amyloidogenesis (14). Because a possible inhibitory effect of the exogenous female sex hormones on amyloidogenesis could have been concealed by the preceding activity of endogenous sex hormones, and because male mice have estrogen and progesterone receptors in various tissues (23-29), we studied the effect of these hormones as well in male mice. However, even under these circumstances neither estrogen nor progesterone administration led to a reduction in splenic amyloid, suggesting that unresponsiveness to exogenous hormones in female mice is not due to a possible preliminary effect of the endogenous female sex hormones.

The lack of a proamyloidogenic effect of testosterone displayed in this study is not surprising and is consistent with its traditional protecting role in inflammation. Thus, the anti-inflammatory qualities of testosterone are thought to play a role in female predominance in lupus (30-34), and rheumatoid arthritis (35-37), as well as in female preference in several different mouse models of inflammation, including interstitial lung disease (32), rheumatoid arthritis (38), cartilage-breakdown (39) and Sjogren-syndrome (40).

Catacholamines are strongly associated with stress, aggression, irritability and vigilance (41-44), features more typical

Table V. Adrenalin may reduce amyloidogenesis in female mice*.

Type of experiment	Duration of experiment (days)	Material injected (intraperitoneal)	Amount administered twice a day	Positive mice of total	Mean amount of amyloid (± SD)	Pvalues*
Study 1 (Casein)	21	Adrenalin	4 µg	17/20	3.00 ± 1.60	
Control 1 (Casein)	21	Saline	0.2 ml	16/17	3.96 ± 1.18	0.047
Study 2 (AEF + $AgNO_3$)	4	Adrenalin	4 µg	9/9	3.30 ± 0.43	
Control 2 (AEF+$AgNO_3$)	4	Saline	0.2 ml	8/8	3.39 ± 0.67	N.S.
Study 3 (AEF + $AgNO_3$)	3	Adrenalin	4 µg	12/14	1.80 ± 1.01	
Control 3 (AEF + $AgNO_3$)	3	Saline	0.2 ml	11/11	2.33 ± 0.98	< 0.1

*Pvalues were computed using the Student t-test.

to male rather than female mice (45, 46). It was therefore expected that male mice, clustered in a cage and undergoing daily insults (injections), would respond by secreting high levels of catacholamine, and that this response, which is known to exert and occasionally be associated with pro-inflammatory activity (47-50), would underlie the gender differences in amyloidogenesis. Surprisingly, however, in female mice receiving adrenalin, splenic amyloid deposition was found to be less abundant than in the control group (Table V). It is therefore possible that the lower propensity for amyloidosis in female mice stems from a protective effect of certain hormones such as adrenalin. Adrenalin may decrease splenic amyloid deposition by non-inflammatory mechanisms to which female mice may be more susceptible, such as splenic shrinkage (51,52), leading to the redistribution of circulating blood and the depletion of white cells from the spleen (53), which in turn deprive the supply of SAA and proteolytic enzymes from the spleen, and lead to the decrement of splenic amyloid in female mice.

In conclusion, in a murine model male amyloidogenesis is quantitatively more pronounced than that in females. Hormonal factors possibly implicated in these findings include cathecholamines (e.g.,adrenalin) that may reduce female amyloidogenesis. As per previous and the current set of experiments, estrogen, progesterone and testosterone probably contribute only negligibly to this variation. However, additional experiments using models involving gonadal ablation with and without relevant hormonal supplement, and using more sensitive amyloidogenesis protocols, are needed to validate our findings. Gender differences in amyloidogenesis in animal models are consistent with male predominance in human reactive amyloidosis and suggest that genetic more than environmental or socioeconomic factors play a major role in this phenomenon.

References

1. HUSBY G: Amyloidosis. *Semin Arthritis Rheum* 1992; 22: 67-82.
2. GERTZ MA, KYLE RA: Secondary systemic amyloidosis: response and survival in 64 patients. *Medicine* 1991; 70 : 246-56.
3. LIVNEH A, ZEMER D, LANGEVITZ P, SHEMER J, SOHAR E, PRAS M: Colchicine treatment of AA and AL amyloidosis. *Semin Arthritis Rheum* 1993; 23: 206-14.
4. YAKAR S, LIVNEH A, KAPLAN B, PRAS M: The molecular basis of reactive amyloidosis. *Semin Arthritis Rheum* 1995; 24: 255-61.
5. LEVIN M, FRANKLIN EC, FRANGIONE B, PRAS M: The amino acid sequence of a major non-immunoglobulin component of some amyloid fibrils. *J Clin Invest* 1972; 51: 2773-6.
6. COHENAS: Proteins of the systemic amyloidoses. *Curr Opin Rheumatol* 1994; 6: 55-67.
7. GRUYS E, SNEL FWJJ: Animal models for reactive amyloidosis. *Bailliere's Clin Rheumatol* 1994; 8: 599-611.
8. GANG N, DRENTH JPH, LANGEVITZ P *et al.*: Activation of the cytokine network in familial Mediterranean fever. *J Rheumatol* 1999; 26: 890-7.
9. GERSHONI-BARUCH R, BRIK R, LIDAR M, SHINAWI M, LIVNEH A: Male sex coupled with articular manifestations cause a 4-fold increase in susceptibility to amyloidosis in patients with familial Mediterranean fever homozygous for the M694V-MEFV mutation. *J Rheumatol* 2003; 30: 308-12.
10. CAZENEUVE C, SARKISIAN T, PCHEUX C *et al.*: MEFV-gene analysis in Armenian patients with familial Mediterranean fever: Diagnostic value and unfavorable renal prognosis of the M694Vhomozygous genotype – genetic and therapeutic implications. *Am J Hum Genet* 1999; 65: 88-97.
11. GREENSTEIN AJ, SACHAR DB, PANDAY AK *et al.*: Amyloidosis and inflammatory bowel disease. A 50-year experience with 25 patients. *Medicine* 1992; 71: 261-70.
12. WITTENBERG GP, OURSLER JR, PETERS MS: Secondary amyloidosis complicating psoriasis. *J Am Acad Dermatol* 1995; 32: 465-8.
13. DAVID J, VOUYIOUKAO, ANSELLBM, HALL A, WOO P: Amyloidosis in juvenile chronic arthritis: a morbidity and mortality study. *Clin Exp Rheumatol* 1993; 11: 85-90.
14. SHTRASBURG S, PRAS M, DOLITZKY M, PARIENTE C, GAL R, LIVNEH A: Pregnancy and amyloidosis: II. Suppression of amyloidogenesis during pregnancy. *J Lab Clin Med* 2000; 136: 314-9.
15. COHEN AS, SHIRAHAMA T: Animal model: Spontaneous and induced amyloidosis. *Am J Pathol* 1972; 68: 441-4.
16. SHTRASBURG S, LIVNEH A, GAL R, PRAS M: Extremely active murine amyloid enhancing factor. *Clin Exp Rheumatol* 1996; 14: 37-42.
17. SHTRASBURG S, GAL R, PRAS M: Crush and smear technique for rapid detection and semiquantitation of amyloid deposition. *Biotech Histochem* 1991; 66: 203-7.
18. SHTRASBURG S, PRAS M, BREZNIAK N, LIVNEH A: Long term effects of amyloid enhancing factor: Clinical and experimental implications. *Clin Exp Rheumatol* 1998; 16: 299-302.
19. SHTRASBURG S, PRAS M, BREZNIAK N, DOLITZKI M, LIVNEH A: Pregnancy and amyloidogenesis: I. Offspring of amyloidotic mice are not predisposed to develop amyloidosis. *J Lab Clin Med* 1999; 134: 168-72.
20. KISILEVSKY R, AXERLAD M, CORBETT W, BRUNET S, SCOTT F: The role of inflammatory cells in the pathogenesis of amyloidosis. *Lab Invest* 1977; 37: 544-53.
21. AXELRAD MA, KISILEVSKY R, WILLMER J, CHEN SJ, SKINNER M: Further characterization of amyloid-enhancing factor. *Lab Invest* 1982; 47: 139-46.
22. SHTRASBURG S, PRAS M, GAL R, SALAI M, LIVNEH A: Inhibition of the second phase of amyloidogenesis in a mouse model by a single-dose colchicine regimen. *J Lab Clin Med* 2001; 138: 107-11.
23. OGAWA S, LUBAHN DB, KORACH KS, PFAFF DW: Behavioral effects of estrogen receptor gene disruption in male mice. *Proc Natl Acad Sci USA* 1997; 94: 1476-81.
24. VIDAL O, LINDBERG MK, HOLLBERG K *et al.*: Estrogen receptor specificity in the regu-

lation of skeletal growth and maturation in male mice. *Proc Natl Acad Sci USA* 2000; 97: 5474-9.
25. OGAWA S, CHESTER AE, HEWITT SC *et al.*: From the cover: abolition of male sexual behaviors in mice lacking estrogen receptors alpha and beta (alpha beta ERKO). *Proc Natl Acad Sci USA* 2000; 97: 14737-41.
26. KORACH KS: Insights from the study of animals lacking functional estrogen receptor. *Science* 1994; 266: 1524-7.
27. COOKE PS, HEINE PA, TAYLOR JA, LUBAHN DB: The role of estrogen and estrogen receptor-alpha in male adipose tissue. *Mol Cell Endocrinol* 2001; 178: 147-54.
28. WERSINGER SR, SANNEN K, VILLALBA C, LUBAHN DB, RISSMAN EF, DE VRIES GJ: Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor alpha gene. *Horm Behav* 1997; 32: 176-83.
29. PHELPS SM, LYDON JP, O'MALLEY BW, CREWS D: Regulation of male sexual behavior by progesterone receptor, sexual experience, and androgen. *Horm Behav* 1998; 34: 294-302.
30. LAHITA RG: The role of sex hormones in systemic lupus erythematosus. *Curr Opin Rheumatol* 1999; 11: 352-6.
31. KANDA N, TSUCHIDA T, TAMAKI K: Testosterone suppresses anti-DNA antibody production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1703-11.
32. SEGGEV JS, SUNDERRAJAN EV, PALOMO T *et al.*: Pulmonary perivascular and interstitial inflammation in MRL/MpJ-lpr/lpr mice. III. Modulation by cyclophosphamid and sex hormones in 4- and 6-month-old animals. *Clin Immunol Immunopathol* 1991; 60: 289-98.
33. ANSAR AHMED S, PENHALE WJ, TALAL N: Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am J Pathol* 1985; 121: 531-51.
34. ROUBINIAN JR, PAPOIAN R, TALAL N: Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. *J Clin Invest* 1977; 59: 1066-70.
35. CUTOLO M: Sex hormone adjuvant therapy in rheumatoid arthritis. *Rheum Dis Clin North Am* 2000; 26: 881-95.
36. CUTOLO M, SERIOLO B, VILLAGGIO B, PIZZORNI C, CRAVIOTTO C, SULLI A: Androgens and estrogens modulate the immune and inflammatory responses in rheumatoid arthritis. *Ann NY Acad Sci* 2002; 966: 131-42.
37. CUTOLO M, BALLEARI E, GIUSTI M, INTRA E, ACCARDO S: Androgen replacement therapy in male patients with rheumatoid arthritis. *Arthritis Rheum* 1991; 34: 1-5.
38. STEWARD A, BAYLEY DL: Effects of androgens in models of rheumatoid arthritis. *Agents Actions* 1992; 35: 268-72.
39. DA SILVA JA, COLVILLE-NASH P, SPECTOR TD, SCOTT DL, WILLOUGHBY DA: Inflammation-induced cartilage degradation in female rodents. Protective role of sex hormones. *Arthritis Rheum* 1993; 36: 1007-13.
40. ROCHAEM, WICKHAM LA, HUANG Z *et al.*: Presence and testosterone influence on the levels of anti- and pro-inflammatory cytokines in lacrimal tissues of a mouse model of Sjögren's syndrome. *Adv Exp Med Biol* 1998; 438: 485-91.
41. GERRA G, AVANZINI P, ZAIMOVIC A *et al.*: Neurotransmitter and endocrine modulation of aggressive behavior and its components in normal humans. *Behav Brain Res* 1996; 81: 19-24.
42. GERRAG, ZAIMOVIC A, GIUCASTRO G *et al.*: Neurotransmitter-hormonal responses to psychological stress in peripubertal subjects: relationship to aggressive behavior. *Life Sci* 1998; 62: 617-25.
43. SGOIFO A, DE BOER SF, HALLER J, KOOLHAAS JM: Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. *Physiol Behav* 1996; 60: 1403-7.
44. COMINGS DE, JOHNSON JP, GONZALEZNS *et al.*: Association between the adrenergic alpha 2A receptor gene (ADRA2A) and measures of irritability, hostility, impulsivity and memory in normal subjects. *Psychiatr Genet* 2000; 10: 39-42.
45. RODGERS RJ, COLE JC: Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. *Physiol Behav* 1993; 54: 729-36.
46. EWALDS-KVIST SB, SELANDER RK, SANDNABBA NK: Sex related copying responses in mice selectively bred for aggression. P*er - cept Mot Skills* 1997; 84: 911-4.
47. RAINER TH, LAM N, COCKS RA: Adrenaline upregulates monocyte L-selectin *in vitro. Resuscitation* 1999; 43: 47-55.
48. KARALIS KP, KONTOPOULOS E, MUGLIA LJ, MAJZOUB JA: Corticotropin-releasing hormone deficiency unmasks the proinflammatory effect of epinephrine. *Proc Natl Acad Sci USA* 2000; 97: 3782.
49. SCHADE R, GOHLER K, BURGER W, HIRSCHELMANN R: Modulation of rat C-reactive protein serum level by dexamethasone and adrenaline – comparison with the response of alpha 2-acute phase globulin. *Agents Actions* 1987; 22: 280-7.
50. ANDRADE-MENA CE: Catecholamines inhibit alpha/beta interferon production induced by lipopolysaccharide. *Regul Pept* 1996; 65: 219-23.
51. ELTZE M: Functional evidence for an alpha 1B-adrenoceptor mediating contraction of the mouse spleen. *Eur J Pharmacol* 1996; 311: 187-98.
52. HURFORD WE, HOCHACHKA PW, SCHNEIDER RC *et al.*: Splenic contraction, catecholamine release, and blood volume redistribution during diving in the Weddell seal. *J Appl Physiol* 1996; 80: 298-306.
53. TOFT P, HELBO-HANSEN HS, TONNESEN E, LILLEVANG ST, RASMUSSEN JW, CHRISTENSEN NJ: Redistribution of granulocytes during adrenaline infusion and following administration of cortisol in healthy volunteers. *Acta Anaesthesiol Scand* 1994; 38: 254-8.