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CHANGES IN THE HAEMATOLOGICAL PARAMETERS OF WISTAR RATS WITH ADJUVANT ARTHRITIS

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SUMMARY

Adjuvant arthritis (AA) was induced by a single intradermal injection of complete Freund's adjuvant (CFA) into the right hind paw of Wistar rats. Haematological studies revealed a decrease in serum iron concentration, haemoglobin concentration and peripheral red blood cell count of AA rats. On the other hand, the number of lymphocytes and monocytes in the differential count were increased by CFA, and total iron binding capacity were increased by both CFA and incomplete FA.

These results indicate that adjuvant arthritis is associated with an anemia which could be suggestive of chronic-disease type anemia.

Key words: adjuvant arthritis, anemia, haematological parameters.

ÖZET

ADJUVANT ARTRİTLİ SIÇANLARIN HEMATOLOJİK PARAMETRELERİNDEKİ DEĞİŞİKLİKLER

Kronik inflamasyon modeli olarak kullanılan sıçan adjuvant artritinde, bazı hematolojik parametreler değerlendirildi. Adjuvant artriti sıçanların sağ arka ayak pençesine Freund's komplet adjuvantı enjeksiyonu ile oluşturuldu. Serum demir düzeyi, hemoglobin konsantrasyonu ve periferik eritrosit değerlerinde bir azalma gözlenmesine karşın, total demir bağlama kapasitesinin ve periferik kan yaymasında lenfosit ve monosit sayılarının arttığı belirlendi.

Bu bulgular gelişmekte olan kronik inflamatuar reaksiyona bir aneminin eşlik ettiğini ve patolojik mekanizması tam olarak belirlenmemiş olan bu aneminin, bir kronik hastalık anemisi tipi olduğunu göstermektedir.

Anahtar Kelimeler: adjuvant artriti, anemi, hematolojik parametreler.

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INTRODUCTION

The rheumatoid arthritis (RA) and antiinflammatory drugs could be evaluated by a number of laboratory methods (1, 2, 3, 4). Arthritis could be induced in sensitive strains of rats, including Wistar rats, by intradermal injection of complete Freund's adjuvant (CFA). This type of arthritis is known as adjuvant arthritis (AA). It is characterized by an intensive inflammatory reaction at the site of injection. This reaction is known as primary reaction (5). Eight to 15 days after CFA injection, the inflammatory lesions and several articular lesions develop in the contralateral paw, leading to a severe polyarthritis and systemic alterations known as secondary reaction (5, 6, 7). Plethora of data indicate that AA involves a cellular immunity besides the chronic inflammatory component of AA (8) and bears some resemblance with RA (5, 9).

AA is one of the most widely used animal model of arthritis in the evaluation of the antiarthritic drugs (5, 6, 10, 11). However, although several parameters of AA have been described, no detailed study on serum iron and ferritin concentration and total iron binding capacity (TIBC) is available in the literature. The present study was therefore undertaken to investigate the changes in the haematological parameters of AA rats.

MATERIAL AND METHOD

Animals. Wistar rats, weighing 150-200 g at the beginning of the experiment were used. The rats were housed under standard laboratory conditions. Food and water were supplied *ad libitum*.

Induction and evaluation of AA. In this study AA was induced according to a previously described method for the evaluation of AA and the effect on by the antiinflammatory drugs (7, 9, 12). Briefly, 0.1 ml of CFA, containing 10 mg/ml of heat-killed *Mycobacterium tuberculosis* suspended in paraffin oil, was injected into the plantar surface of the right hind paw. The inflammatory reaction was evaluated by measuring the volume of both hind paws plethysmometrically (8, 13). The rats were divided randomly into three groups of 10 rats each. The rats in group 1 received normal saline. In group 2, the rats were injected with 0.1 ml of incomplete Freund's adjuvant, IFA (85 % paraffin oil and 15 % mannide monooleate). The rats in group 3 received 0.1 ml of CFA and were regarded as AA rats. The paws volumes were measured before and after injection of CFA (this is regarded as day 0). Thereafter, the volumes were measured on days 4, 8, 11, 14, 18 and 24th day after injection of CFA. Body weights were also measured before each plethysmometric measurement.

Haematological studies. Under light ether anesthesia, blood samples of 3 ml were drawn directly from heart at the end of the experiment (14). Haemoglobin concentration, total number and differential count of white blood cells (WBC) and red blood cells (RBC) were determined. Serum iron was quantified using colorimetric Ferrozine method (Sclavo, Italy). TIBC was also quantified by a modified Ransey

Table I. Changes in right and left hind paw volumes (ml) induced by CFA in rats

Day	Right hind paw volume		Left hind paw volume	
	IFA	CFA	IFA	CFA
0	0.410±0.01	0.472±0.07	0.410±0.01	0.472±0.01
4	0.549±0.02	1.212±0.06*	0.410±0.01	0.491±0.08
8	0.549±0.02	1.033±0.05*	0.410±0.01	0.512±0.01*
11	0.529±0.02	1.024±0.07*	0.410±0.01	0.536±0.01*
14	0.529±0.02	1.068±0.08*	0.410±0.01	0.561±0.01*
18	0.536±0.02	1.123±0.09*	0.410±0.01	0.605±0.04*
24	0.543±0.01	1.098±0.09*	0.410±0.01	0.611±0.05*

Values are mean ± S.E. of increases from the basal volume of either paw, 1.18±0.02 ml (Mann-Whitney U test): * $p<0.05$, CFA vs IFA in the corresponding day.

technique (Sclavo, Italy). Ferritin was determined using Coat-A-Count Ferritin IRMA-Solide-phase immunoradiometric assay (DPC, Diagnostic Products Corporation, LA, USA).

The animals with AA were euthanized at the end of the experiment because of hypovolemia following cardiac puncture. Moreover, the rats were with severe arthritic inflammation intensified by time, and their body weights continued declining because of either depressed appetite or the pain-induced suppressed food intake.

Statistical analysis. Mann Whitney-U test and Wilcoxon signed ranks test were used to evaluate inter- and intra-group changes respectively.

RESULTS

Changes in right and left hind paw volume. Table I shows that in right hind paw (but not in the left one) IFA significantly increased the volume ($p<0.05$; Wilcoxon signed ranks test, n=10). The effect was evident from day 0 and persisted along the experimental period.

CFA increased the volume of right hind paw on the day 0 ($p<0.05$; Wilcoxon signed ranks test, n=10). The effect of CFA was increased from day 0 on. This effect was significantly greater than the effect of IFA from day 4 on ($p<0.05$; Mann-Whitney U test, n_1 and n_2=10). Moreover, CFA increased the volume of left hind paw from day 8 on ($p<0.05$; Wilcoxon signed ranks test, n=10). The latter effect was also significantly higher than IFA ($p<0.05$; Mann-Whitney U test, n=10).

Changes in body weight. Table II shows that body weight was significantly increased in the IFA group ($p<0.05$; Wilcoxon signed ranks test, n=10) whereas it was significantly decreased in the CFA group ($p<0.05$; Wilcoxon signed ranks test, n=10) at the end of the experiment. Moreover, the reduction in the body weight of CFA rats was significantly more than of IFA rats from day 8 on ($p<0.05$; Mann-Whitney U test, n_1 and n_2=10).

Table II. Changes in body weight (gram) caused by AA induced by CFA in rats.

Days	IFA	CFA
0	163.33±4.59	168.33±8.23
4	167.50±5.28	157.50±7.82
8	167.50±5.28	146.66±5.72*
11	169.16±4.55	146.66±5.72*
14	170.83±5.06	140.83±6.76*
18	170.00±4.28	140.00±7.41*
24	170.83±5.06	140.00±7.41*

Values are mean ± S.E. Statistically significant differences (Mann-Whitney U test): * $p<0.05$ vs IFA.

Haematological parameters. Table III shows that AA was accompanied by a significant reduction in haemoglobin and RBC ($p<0.001$; Mann-Whitney U test, n_1 and $n_2=10$). Total WBC count of both IFA and CFA rats did not significantly change. However, in CFA rats the lymphocytes were significantly increased, whereas the polymorphes (neutrophils) were significantly reduced ($p<0.05$; CFA vs IFA, Mann-Whitney U test, n_1 and $n_2=10$). The monocytes were not changed in IFA- and CFA-rats.

Table IV shows that AA rats had a decreased serum iron concentration as compared with IFA rats ($p<0.01$; Mann-Whitney U test, n_1 and $n_2=10$). TIBC was increased in both AA and IFA groups ($p<0.05$; Mann-Whitney U test, n_1 and $n_2=10$). No significant change was obtained in serum ferritin levels ($p>0.05$; Mann-Whitney U test, n_1 and $n_2=10$).

DISCUSSION

The development of the primary and secondary reactions observed in this study is consistent with the results described previously. These changes are known to be accompanied by haematological changes of chronic inflammatory and immunological type (5, 6, 7, 8, 12, 15). Moreover, the secondary reaction was accompanied by a reduction in body weight from the day 8 on. This result could be ascribed to the pain that accompanied AA. The possibilty of inability of the rats to get access to the food is excluded because the food was placed on the floor of the cages at the easy excess of the rats.

Data concerning the haematological changes in AA are inconsistent. WBC are reported to be increased (9, 16) or not changed (17). The latter result is in line with our findings, as we obtained no significant change in total WBC count. Moreover, lymphocytes and neutrophils were also reported to be not changed (18) or to be increased (17). In this study, the lymphocytes were increased while the neutrophils were decreased. This may indicate that AA involves a chronic inflammatory and/or immunological components. The inconsistency of the data among various research

Table III. Haematological changes in rats.

	Control	IFA	CFA
Haemoglobin (g/dl)	11.96±0.21	11.83±0.41	10.17±0.32**
RBC ($x10^6/mm^3$)	5.42±0.49	4.95±0.35	2.52±0.14**
Total WBC ($x10^3/mm^3$)	6.38±0.71	5.75±0.65	5.44±0.66
PMN[a] (%)			
Neutrophils	42.05±2.31	42.08±5.41	34.82±4.93*
Eosinophils	3.00±0.65	2.75±0.48	2.82±0.16*
Basophils			
Lymphocyte (%)	51.75±2.91	55.21±4.84	59.68±4.54*
Monocyte (%)	3.25±0.68	2.92±0.51	4.68±0.99

[a] PMN: Polymorphonuclear leukocyte. Values are mean ± S.E. Statistically significant differences (Mann-Whitney U test): * $p<0.01$, ** $p<0.001$ vs IFA.

groups could be ascribed to variation in the method of AA induction and animal species.

AA is known to be accompanied by an anemia involving reduced haemoglobin, RBC and serum iron concentration (9, 19, 20, 21). AA seems to be similar to RA in this regard (17, 22, 23). Our results also indicated that anemia was involved in AA. Haemoglobin, RBC and serum iron were decreased in AA rat. This type of anemia could be an anemia of chronic disease type rather than iron deficiency anemia, because serum ferritin levels were not changed, whereas TIBC was increased in IFA and CFA rat. However, other causative factors should also be considered, such as disturbance of release of iron from its stores due to an extracorpuscular haemolytic factor (22, 24) and deficiency in erythropoiesis dependent on T lymphocytes which may suggest contribution of an immune process to the development of anemia in RA (25).

In summary, the present results revealed that AA induced an anemia of chronic disease type, suggesting a similarity with RA of humans. Further haematological and immunological studies are going to be conducted to determine the pathological changes underlying AA-anemia of rats.

Table IV. Changes in serum iron, total iron binding capacity (TIBC) and serum ferritin in rats with adjuvant arthritis.

Groups	Serum iron (μg/dl)	TIBC (μg/dl)	Serum ferritin (ng/ml)
Control	206.8±21.5	392.8±37.4	1.05±0.100
IFA	190.6±4.6	501.2±21.3*	0.84±0.001
CFA	117.0±10.5**	528.0±21.6*	1.21±0.050

Values are mean ± S.E. Statistically significant differences (Mann-Whitney U test): * $p<0.05$, ** $p<0.01$ vs control.

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