Bull Yamaguchi Med Sch 35(1-2) : 1-5, 1988

Age-dependent Variations in Chemiluminescence Response of Rat Whole Blood

Yoshio Kimura, Junichi Mise**, Yoshihisa Fujikura*** and Tetsuo Fukumoto****

*Department of Internal Medicine Shimonoseki National Hospital, Shimonoseki, Yamaguchi 751, Japan
**Yamaguchi Research Institute of Gerontology, Shimonoseki Yamaguchi 751, Japan and
***Department of Anatomy, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan
(Received January 29, revised May 6, 1988)

Abstract Chemiluminescence (CL), one of the assay system for phagocytic functions of leukocytes, especially of polymorphonuclear leukocytes was used for assessing the age-related changes of immunocompetence in rat. The estimation of CL was carried out using freshly drawn unfractionated whole blood. The peak CL responses in newborns and relatively old rats were low compared to those in adult rats, although only the difference in the newborn group was statistically significant. The kinetic properties of CL response of newborns were also different from those of other age groups of rats. These results suggest the age-related changes of phagocytic functions may exist which may contribute to the high susceptibility to infection of old animals and newborns.

Key Words : Polymorphonuclear leukocyte, Phagocytic function, Chemiluminescence, Rat, Aging

Introduction

The age-related changes of immunocompetence are well-documented in experimental animals[1] and in humans.[2)3)] It is a common belief, based mostly on clinical experience, that resistance to infection is lower in old individuals than in young adults. In view of this, we studied a phagocytosis-associated function, the chemiluminescence (CL) of polymorphonuclear leukocytes (PMN) during the aging process.

In most studies on CL responses[4], purified and washed PMNs have been used. This method requires a large volume of blood, is time consuming, and may not reflect the *in vivo* situation. In this study the estimation of CL was carried out using freshly drawn unfractionated whole blood[5)6)] in order to study PMN functions under reasonably natural conditions.

Materials and Methods

Experimental animals

Rats were divided into the following experimental groups according to the age in months ; (group I ; newborn, group II ; 1-6 months, groupIII ; 7-12 months, groupIV ; 13-18 months and groupV ; 19-24 months). We used only female rats. Blood samples were collected in heparinized tubes from the abdominal aorta under ether anesthesia. In the case of newborn rats, blood was obtained from the heart. Sexes of newborn rats were not determined.

Chemiluminescence assay

Luminol (Aldrich Chemical Company, USA) was prepared as a 1 mM stock solution in HEPES buffered saline solution (HBSS), which was then passed through a Millipore filter (0.45 μm). Zymosan (Tokyo Kasei Co. Ltd., Japan) was suspended in HBSS to a concentration of 40 mg / ml. For CL measurements, heparinized

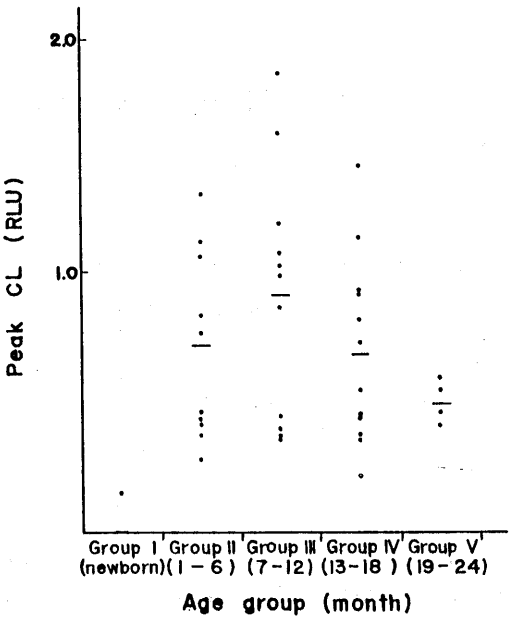

Fig. 1 Peak CL responses of various age groups. The peak CL response (RLU) of individual rats, were plotted against the groups of ages. Mean values of each group were indicated by horizontal bars.

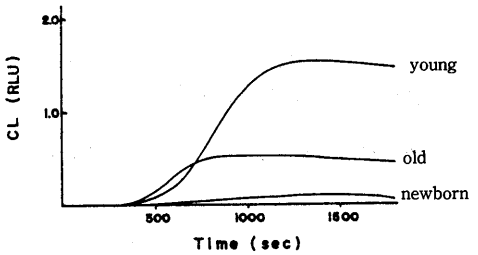

Fig. 2 Time courses of whole blood CL response of rats each representing newborn, young or old groups. Whole blood of rats : newborn, rat no. 1 ; young, rat no.22 in group III ; old, rat no. 38 in group V.

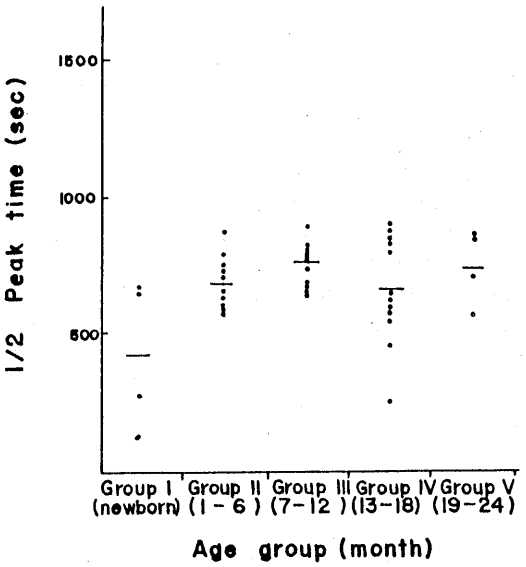

Fig. 3 Half peak times of CL response were plotted against the groups of ages. Mean values of each group were indicated by horizontal bars.

whole blood (0.2ml) was diluted with HBSS (1.2ml), The samples to be analyzed were mixed with luminol (0.4ml) after which zymosan (0.1ml) was added. Immediately CL was monitored for 30 minutes with a TC-4000 lumiphotometer (Labo Science, Japan). The maximum height of CL response (peak CL response) was measured and expressed as the relative light unit (RLU).

Statistical analysis

Student's t-test was used for the statistical analysis.

Results

1) *Whole blood CL responses of various age groups*

Table 1 shows the results of whole blood CL responses of individual animals grouped by age, which demonstrated the tremendous variations in peak CL. Fig. 1 shows a summary of the peak CL responses of 5 groups. A depression in the peak CL response was remarkable in newborns when compared with adults. The mean peak CL responses in group I (newborns), group II (1 to 6 month), group III (7 to 12 month), group IV (13 to 18 month), and group V (19 to 24 month) were 0.06, 0.71, 0.91, 0.69 and 0.50 RLU, respectively. The CL responses of newborns were statistically lower than those of other groups ($p<0.01$). In older rats (group V) CL responses were lower than those in younger ones (groups II, III and IV), but the difference was statistically not significant.

Table 1 Summary of whole blood CL responses of various age groups of rats

	Rat No.	Age (month)	Peak CL* (RLU)	Peak time** (sec)	WBC (cells/mm³)	Neutrophil (%)
Group I	1	newborn	0.033	254	ND	ND
	2	newborn	0.032	1327	ND	ND
	3	newborn	0.157	560	8300	25.5
	4	newborn	0.034	1374	15000	41.0
Group II	5	1	0.283	1585	3600	14.0
	6	1	0.459	1162	6400	19.5
	7	2	0.375	1148	7000	ND
	8	2	0.431	1310	11000	10.0
	9	2	0.419	1438	6800	55.5
	10	2	1.194	1172	9300	44.5
	11	5	0.837	1432	6900	18.5
	12	6	1.063	1498	11000	41.0
	13	6	0.759	1757	11300	44.5
	14	6	1.296	1252	7600	30.5
Group III	15	8	1.026	1651	6700	40.0
	16	8	0.989	1354	9000	22.0
	17	10	1.753	1335	11600	26.5
	18	10	0.369	1570	5200	12.5
	19	11	0.860	1795	8800	47.5
	20	11	0.394	1377	6200	47.0
	21	12	1.079	1314	7000	42.0
	22	12	1.536	1479	10200	54.0
	23	12	1.182	1612	9500	36.5
	24	12	0.378	1637	5000	50.0
	25	12	0.449	1566	4100	38.5
Group IV	26	14	0.540	514	7800	16.0
	27	14	0.219	1701	7900	33.0
	28	15	1.130	1091	9200	53.5
	29	15	0.931	1292	7200	6.5
	30	15	0.354	1280	5700	25.5
	31	15	0.814	1732	6700	45.5
	32	15	0.444	1799	7800	49.5
	33	16	0.414	1182	9900	12.5
	34	16	0.920	1626	10800	26.0
	35	17	1.405	1156	8400	ND
	36	17	0.727	915	5200	35.0
	37	17	0.372	1791	6900	33.0
Group V	38	19	0.403	1151	4200	ND
	39	20	0.454	1684	8600	55.5
	40	20	0.591	1705	10300	41.5
	41	23	0.542	1426	4300	30.0

* The maximum height of CL response,

** The time required for maximal CL response, ND ; not determined

2) *Kinetics of CL response*

The time required for the maximal CL response (peak time) was determined on individual rats (Table 1). In Fig. 2 are shown the kinetics of CL responses of representative rats from newborn, young and old groups. The kinetics was rather similar for young and old rats, but not for newborns. To analyze further the kinetics of CL response, the time required for half maximal CL (1/2 peak time) of individual rats was determined (Fig. 3). The 1/2 peak time of the newborn group was shorter than those of other groups, although the difference was statistically not significant.

Discussion

In the present study, whole blood was used for CL measurements. The advantages of this method are speed, easiness, as well as ability to measure phagocytic functions of PMN and opsonic activity of serum simultaneously under reasonably natural conditions. The results from this study demonstrate that whole blood CL is depressed in the age extremes : newborns and very old rats. The low levels of whole blood CL response associated with the age extremes could contribute, at least in part, to a high incidence of infectious diseases in young and old individuals. The low levels of whole blood CL of newborns may be due to immaturity of several factors which include phagocytosis, hexose monophosphate shunt activity, and serum opsonic activity. The influence of RBC present in the assay system should also take into consideration. Since our findings were essentially similar to those of Van Epps *et al.*[7] (1978) who measured CL response before and after RBC lysis, RBC contamination could not account for depressed CL response in newborns. Miller[8] (1971) reported that both membrane deformability and chemotaxis of PMN were low in neonatal cells and suggested that the cellular defects in phagocytosis and chemotaxis found in the neonatal PMN might be related to developmental immaturity of common biologic mechanisms. In addition, Tonooka *et al.*[5] (1983) demonstrated that low opsonic activity caused low CL response.

Many investigators have demonstrated an age-related decline in the immune system of both experimental animals[1] and humans.[3][4] Antonaci *et al*[9] (1984) reported, concerning PMN functions, that phagocytosis, NBT reduction and chemotaxis were reduced, while nylon fiber adherence was normal during the aging process. They presented evidence for a decreased O_2-production by aged monocyte and PMN. In contrast, recent studies by Sakane[10] (1987) indicated that phagocytic activity and production of active oxygen (O_2^-, H_2O_2, OH^-) and chemiluminescence by neutrophils from young and old donors were comparable, but chemotaxis of old neutrophils was defective. The estimation of CL in those studies was carried out using purified neutrophils while we used whole blood. An explanation for the discrepancy between our results and the data by Sakane may be due to the difference in the techniques which were employed. In conclusion, although further studies are needed to understand fully the CL response in young and old rats, our studies suggest that the defects of CL response may be an important factor of high susceptibility to infections in old people and newborns.

The authors would like to express our thanks to professor E. L Cooper, Department of Anatomy, School of Medicine University of California, Los Angeles, California, USA for his criticism on preparing this manuscript and Mr. M. Tamechika, Department of Anatomy, Yamaguchi University, for his technical assistance.

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