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Complement, Circulating Immunocomplexes and Autoantibodies after Ionizing Radiation Exposure

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The short- and long-term effects of whole body irradiation by a single dose (4Gy) on some immunological parameters in mice have been studied. The results showed a transitory but significant impairment of classical activity of complement, high incidence of anti nuclear, anti gastric parietal cells and anti smooth muscle autoantibodies, and circulating immunocomplexes in sera on irradiated mice. These immunological changes could be related to complications developed as late manifestations of radiation effects.

Key words: CH50, Complement, Autoantibodies, autoimmunity, Circulating immunocomplexes, Irradiation.

Ionizing radiation is often used in the clinic as part of the preparation for bone-marrow transplantation and in treating of certain kinds of cancer. However, exposure of an organism to this type of radiation can lead to a series of physiological and morphological changes that constitute the acute radiation syndrome. Little is known about the effects of radiation on the immune system. Most of the published studies refer to the alterations that occur in the activity of immunological cells, and it has been reported that both the proliferative response of lymphocytes and their capacity for synthesizing immunoglobulins are activated by radiation (1, 2).

Particularly little is known about the effects of ionizing radiation of humoral immunity parameters, like the activity of the classic complement pathway or generation of autoantibodies and circulating immunocomplexes. DE-EN *et al.* (3) reported that patients undergoing radiotherapy show an increase in serum levels of autoantibodies and immunocomplexes. However, the pathologies observed in these patients make it difficult to evaluate

the results obtained. This, in turn, points to the need for an experimental model for studying humoral immunity in healthy organisms subjected to ionizing radiation.

The aim of the present study was to examine the long and short-term effects of a single dose (4Gy) to ionizing radiation on certain humoral immunity parameters in healthy mice.

Materials and Methods

Animals. — Healthy 6 month old BALB/c mice were used.

Radiation. — The animals were placed in plastic containers and were exposed to a single dose of 4Gy ionizing radiation in a linear Sagittaire accelerator at a rate of 2 Gy/min.

Sera. — After irradiation the animals were divided into groups which were killed 0, 15, 30 and 90 days later. After removing the animals blood by heart puncture, the blood was left to coagulate for 1 h at 37 °C and centrifuged. The serum was then removed and frozen at −70 °C until use.

Classic complement pathway. — The activity of the classic complement pathway was measured using a haemolytic assay of rabbit red blood cells sensitized with guinea pig hemolysin, according to the method of TANAKA *et al.* (14). The number of hemolytic units was determined from the degree of hemolysis, by the MAYER method (9).

Circulating immunocomplexes. — The presence of circulating immunocomplexes (CIC) in serum was detected using the anticomplementary activity method. Immunocomplexes in serum were precipitated with polyethylen glycol and the capacity of the precipitate to consume a known quantity of complement was measured, following described methods (8, 10).

Autoantibodies. — The presence of autoantibodies in serum was determined by indirect immunofluorescence. Section of rat liver, kidney and stomach were used to examine antinuclear (ANA), anti smooth muscle (ASMA), anti mitochondrial (AMA) and anti gastric mucosa parietal cells (GPC) autoantibodies. In addition, *Crithidia luciliae* preparations were used to study anti native DNA autoantibodies (DNAn). In all cases, anti mouse immunoglobulin antiserum conjugated with fluorescein (Ortho) was used.

Results

Classic complement pathway. — As is shown in table I, there was a decrease in the activity of the classic pathway immediately after irradiation. Activity increased thereafter, reaching its maximum 15 days after exposure to radiation. In then decreased until it again reached normal levels.

Circulating immunocomplexes. — Immediately after exposure to radiation, the anticomplementary activity observed in irradiated mice did not differ from that of controls, but it then increased, reaching

Table I. *Classic complement activity (U CH50/ml) and CIC level (% CH50 consumed).* The arithmetic mean and standard desviation are expressed. * $p < 0.01$ (Mann-Whitney test).

Mice group	N	CH50	CIC
Controls	14	6.23 ± 4.24	2.86 ± 3.93
0 days	7	3.70 ± 2.76	2.80 ± 2.26
15 days	12	14.02 ± 7.93*	46.30 ± 4.76*
30 days	14	8.05 ± 6.54	38.70 ± 6.30*
90 days	10	6.10 ± 3.66	29.90 ± 9.30*

Table II. *Autoantibodies in serum.* The number of positive cases is given.

Mice group	N	ANA	ASMA	AMA	GPC	DNAn
Controls	14	0	1	0	0	0
0 days	7	0	0	0	0	0
15 days	12	2	10	0	3	0
30 days	14	1	11	0	0	0
90 days	10	0	8	0	2	0

maximum value 15 days after exposure to radiation. It decreased progressively thereafter, but was still above normal values 90 days after exposure.

Autoantibodies. — From day 15 after exposure to radiation, a high rate of autoantibodies was observed (table II). The autoantibodies present were ANA, ASMA and GPC. Animals with ANA antibodies showed no DNAn antibodies.

Discussion

The present study shows that ionizing radiation induces certain long and short-term immunological alterations that affect some immunological parameters in the serum of irradiated animals.

The transitory inhibition of serum CH50 activity was followed by a progressive increase in this parameter. The origin of this increase, also transitory, is unknown but may be related to the increased activity observed in some immunological cells, like monocytes and macrophages. It has been demonstrated that when these cells are subjected to ionizing radiation, an increase in protein synthesis occurs. Among the proteins thus affected are some components of the classic component pathway, and this, in practice, means a transitory increase in CH50 activity (4).

The rise in the activity level of the classic complement pathway has no important effects on the organism. Of greater interest, therefore, are the implications of its consumption, which takes place later. This consumption is probably due to the increase in the serum concentration of circulating immunocomplexes, manifested by an elevated anticomplementary activity. It is well known that immunocomplexes activate and consume the classical complement pathway, thus producing a decrease in CH50 (10, 13). This activation and consumption lead to the production of anaphylotoxins capable of triggering an inflammatory response that could be related to certain vascular alterations described in irradiated animals (6, 7). It has also been demonstrated that the hydroxyl radicals generated after exposure to radiation or thermic injury can activate the complement, thus contributing to the decrease in the CH50 value (11).

In any case, the level of circulating immunocomplexes in serum is generally considered an indication of the alterations produced in the immune system by injury.

The presence of autoantibodies in serum, demonstrated in the present study, indicates that the immunocomplexes could have an autoimmune origin. The formation of autoantigens may be a consequence of the cell death caused by radiation, which releases internal antigens into the extracellular space. The production of autoantibodies may also be helped by the decrease in the activity of supressor T cells, which, as has been demonstrated previously, are highly radiosensitive (5, 12).

In conclusion, then, the present study demonstrates that ionizing radiation at a dose of 4Gy can significantly alter the classical complement activity and generate circulating immunocomplexes and autoantibodies. These alterations seem to depend on the time that passes from the moment of radiation, and they disapear or decrease after three months.

Resumen

Se investigan los efectos que produce sobre algunos parámetros del sistema inmune del ratón a corto y largo plazo, la irradiación total del cuerpo con una dosis simple (4Gy) de radiación ionizante. Los resultados muestran un aumento significativo pero transitorio, en la actividad de la vía clásica del complemento, y una mayor incidencia de autoanticuerpos anti nucleares, anti músculo liso y anti células parietales, así como de inmunocomplejos circulantes. Estas alteraciones inmunológicas podrían estar relacionadas con el desarrollo de las complicaciones que se manifiestan a consecuencia de la radiación.

Palabras clave: CH50, Complemento, Autoanticuerpos, Autoinmunidad, Inmunocomplejos circulantes, Irradiación.

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