SPECIFIC SUBSTANCES FOR DIAGNOSIS AND THERAPY OF RADIATION INJURY

Viacheslav Maliev¹ *, Vidmantas Bižokas², Dmitrij Popov³, Liudmila Malieva⁴, Nikolai Lysenko⁴, Albina Aniulienė²

¹Department of Biotechnology of Scientific Center of the Russian Academy of Sciences
Vladikavkaz, North Ossetia-Alania, per. Keramiceskij 4, Russia
²Department of Non-Infectious Diseases of the Veterinary Academy of the Lithuanian University of Health Sciences
Tilžės 18, LT-47181 Kaunas, Lithuania
³OGP Technologies Inc., 9 Marathon ave., L4K5G9, Concord, Ontario, Canada
⁴Moscow State Academy of Veterinary Medicine and Biotechnology, Academician Skriabin 23, Moscow, Russia

*Corresponding author. Present address:
Department of Biotechnology of Scientific Center of the Russian Academy of Sciences
Vladikavkaz, North Ossetia-Alania, per. Keramiceskij 4, Russia; e-mail: niobiot@mail.ru

Abstract. Radiation toxins induce high toxicity reactions after exposure. Acute Radiation Disease (ARD) or Acute Radiation Syndromes (ARS) are defined as the collective toxic clinical states observed from the acute pathological processes in various doses in irradiated mammals.

Multifactor fundamental research under experimental and industrial conditions on farm and laboratory animals (parabionts, gnotobionts, mice, rats, rabbits, dogs, sheep, pigs, cattle, and horses), and also on humans (blood serum of Chernobyl NPP clean-up workers) conducted between 1982 and 2002 enabled us to establish the existence of two previously unknown phenomena:

• the phenomenon of reversible redistribution of cytobiochemical parameters in the blood-interstices-lymph-blood system of irradiated animals, which supports compensatory maintenance of homeostasis;
• high molecular mass glycoprotein (molecular mass-200–250 kDa) – radiation antigens in the lymphoid system with epitopes specific to each form of radiation sickness 1, 2, 3, 4, after animals have been irradiated in doses inducing the development of the cerebral (1), toxic (2), gastrointestinal (3) and typical (4) forms of acute radiation sickness.

These two phenomena allowed us to develop a methodology for producing specific means of prevention, diagnosis, and treatment of radiation sickness (know-how) with antiradiation vaccine, a serum and a set of diagnostics for conducting ELISA analyses.

Keywords: radiation antigens, radiation injury, diagnostics, therapy.

SPECIFINĖS RADIACINIŲ PAŽEIDIMŲ DIAGNOSTIKOS IR TERAPIJOS MEDŽIAGOS

Viacheslav Maliev¹ *, Vidmantas Bižokas², Dmitrij Popov³, Liudmila Malieva⁴, Nikolai Lysenko⁴, Albina Aniulienė²

¹Mokslo centro Biotechnologijos departamentas, Rusijos mokslų akademia
Vladikaukazas, Keramičeskij skg. 4, Šiaurės Ossetija-Alanija, Rusija
²Neužkrečiamųjų ligų katedra, Veterinarijos akademia, Lietuvos sveikatos mokslų universitetas
Tilžės g.18, LT-47181 Kaunas
³OGP Technologies Inc., 9 Marathon ave., L4K5G9, Konkordas, Ontario, Kanada
⁴Maskvos K. I. Skriabino vaistininkų veterinarijos medicinos ir biotechnologijos akademia
Akademiko Skriabino 23, Maskva, Rusija

Santrauka. Daugiafaktoriiniai ukinės paskirties ir laboratorinių gyvūnų (parabiontų, gnotobiontų, pelių, žiurkių, trišų, šunų, avų, kiaulų, galvijų ir arklų) bei žmonių, dirbusių Černobylio AE 1982–2002 metais, kraujo sukurto tyrimai leidžia teigti, kad egzistuoja nežinomi du reiškiniai: 1) apšvitintų gyvūnų kraujo-impociacijumo-limfmosis kraujų sistemos citocheminių rodiklių grįžtumas perskirstymas ir 2) specifinės immunomokslės reakcijos į radiobiologinį poveikį, apimantį didelės molekulinės masės (200–250 kDa) glikoproteinio formavimąsi, pasireiškiančią specifiniams radiacinėms ligoms cerebrinei, toksinei, skrandžio-žarnų ir tipinei formoms.

Vadovaujantis nustatytais reiškiniais sukurta metodika, leidžianti praktiškai išvengti specifines spindulinės ligos prevencijos, diagnostikos ir gydymo (know-how) priemonės.

Raktajodžiai: radiaciniai antigenai, radiaciniai pažeidimai, diagnostika, gydymas.

Introduction. Literary sources and patent search attest the fact that when any living system is irradiated, the induction mechanism of the radiobiological effects is a function of the synergism of the direct effect of radiation and the biologically active radiotoxic substances that form, the efficacy of which are a function of the duration and dose of irradiation. These studies formed the basis for the methods of diagnosis, prevention and treatment of
acute and chronic radiation injuries in use today and were nonspecific with respect to the pathogen-forming agent. However, polyclonal experimental studies of farm and laboratory animals have unambiguously shown that the immunoneurochemical processes that occur in the lymphoid system are definitive with respect to pathogenesis of the radiobiological effect and are specific to level of radiation over a wide spectrum of values.

Comparative logical analysis of the extensive base of data that had been obtained by well-known radiobiological schools and groups of scientists in related scientific areas in the United States, Germany, France and Japan served as the theoretically-based prelude to this study. We will consider here the most significant fundamental radiobiological research studies. In particular, A.M. Kuzin et al (1973, 1987) isolated regakgenon from plant tissue. This substance has a radioimmunizing effect on cell genomes, accompanied by depression of DNA synthesis, decreased cell division and tissue growth, formation of pyknotic nuclei, chromosome fragmentation, and formation of mutants. At the same time, after administration of regakgenon and other radiotoxins isolated from irradiated tissues, animals failed to display the entire "bouquet" of radiation reactions (symptoms of acute radiation sickness of various forms and severity could not be modeled in experimental animals). The broad spectrum of ordinary chemical compounds that can have analogous radiomicrometric effects to those of regakgenon demonstrates the lack of specificity in induction of these effects by the main classes of radiotoxins: hydroperoxides, peroxides, polyphenols, semiquinones and quinines, ketocarboxydehydes, biogenic amines, proteins and polypeptides. These compounds are always present in an untreated animal and play a role in the biochemical processes of catabolism and anabolism that maintain homeostasis. Radiation exposure induces a process in which the levels of one or another class of these compounds increase.

The studies of Kopylov et al. (1992), Wong, Van der Kogel (2004) demonstrate the critical importance of eliminating quinoid radiotoxins from the body in treatment by hemosorption of animals after lethal doses of gamma irradiation.

Vladimirov et al. (1993) detected a fraction of low molecular weight DNA in the blood plasma of an animal exposed to ionizing radiation. There are two points of view on the origin and role of this DNA. One of them links its origin with the postradiation fragmentation of chromatin from lymphocyte nuclei. The other point of view postulates an active process serving to transmit a "horizontal" flow of genetic information and to correct cell functioning by means of intragenome restructurings.

Evolutionary phylogenesis of the lymphoid system in animals (from the simplest organisms to mammals), which evolved in the presence of a constant radiation factor, made it possible to integrate the following eight vital functions: immunological, metabolic, transport, storage, hematopoiesis, resorption, excretion and barrier-filtration (Aleksyeyev et al., 1985; Bizokas, Malijev, 2001; Kupryanov, 1983; Levin, Yu, 1986; Panchenkov et al., 1970; Rusnyak et al., 1957; Kleining, Vink, 2009; Epperly et al., 2011).

There are very many studies of the radiation pathology of lymphoid organ structure and function in mammals (Aksyantsev et al., 1964; Bardychev, Tsyb, 1985; Belousova, Fedotova, 1968; Ivanov et al., 1981; Gruzdev, 1988; Kirshin, Belov, 1986; Malijev, 1984, 1992, 1997; Yarin, 1981; Yarmenenko, 1984; Anderson et al., 1974; Kuznetsova, 1989; Berger, 2006; MacCann, 2006; Popov, Maliev, 2010). They contributed to disclosure of certain mechanisms underlying the essential immunodepressive effect of ionizing radiation, emphasizing the death of interphase lymphocytes (Petrov et al., 1970). The reproductive death of lymphocytes also makes some contribution to this immunodepressive mechanism (Kudryashov et al., 1982).

These authors started from the fact that an important proof of the decisive role of lymphocyte deaths in radiation lymphopathology is the correspondence of the RD, obtained when the whole body is immunized, RID=0.8 Gy, and that in the system of adaptive transfer of lymphoid cells, RD=0.7 Gy. This a priori circumstance convinced radiobiologists that the interphase and reproductive death of lymphocytes is decisive in the development of radiation immunopathology (which we do not find incontrovertible, since this occurred before our discovery of the phenomenon of reversible redistribution of biochemical parameters in the blood-interstices-lymph-blood system of irradiated animals).

Furthermore, B. Zhivotovskiy (1988) demonstrated a quantitative association between the pyknotic changes in the cell nuclei of thymocytes and production of products of postradiation chromatin decay. The enzyme responsible for the decomposition of chromatin in irradiated cells is Ca/Mg-dependent endonuclease. The areas of endonuclease attack are distributed randomly and are not associated with the level of repetition of nucleotide sequences of DNA or the transcriptional activity of chromatin. This supports the conclusion that the radiation death of lymphoid cells and the general biological phenomenon of programmed cell death are identical in nature. Ionizing radiation, thus, like other lympholytic factors, can evoke induction of groups of specific genes that switch on the cell death program. However some researchers (Zhivotovskiy, 1988; Khashan et al., 1988; Wataneko, McVitte, 2004; Elinger, Thomson, 2004; RaStogi, 2009), who, including ionizing radiation in the group of lympholytic agents, without sufficient justification, argue that glucocorticoids, eAMP and alkylating compounds, which are so different in chemical nature, must enter into specific chemical interactions of the epitope-idiotope type with the specific gene loci responsible for cell death, while ionizing radiation by nature is incapable of directly entering into such interaction without adductor-mediators specific to radiation. Thus, we have postulated that high-energy irradiation, like other genotoxins, can evoke the induction of specific genes to switch on the cell death program only if mediated by a system of specific aductors. These hypotheses have been confirmed in our research, as well
as elsewhere.

Studying the trigger mechanism for radiation-induced lymphocyte death, Sorokina (1988) used the results of numerous experiments to show that ionizing radiation induces changes in the antigen phenotype of immature thymocytes in mice. This has the same type of effect as chemical differentiation inductors and thymotropin, which indirectly attests to the specific modifying effect of ionizing radiation.

Fundamental attainments in molecular radiobiology have fostered significant progress in explaining the mechanisms of this process and this has permitted K. P. Khanson (1988), Riedl (2004), Molla, Panes (2007) to approach understanding of many aspects of the occurrence, development and realization of radiation effects in lymphocytes. Within the context of the proposed hypothesis, the interphase death of irradiated lymphocytes is considered one of the examples of the general biological phenomenon of programmed cell death. The interphase cell death occurs as a result of switching on a specific genetic program, the triggering of which, the author asserts, may occur as a result of many signals, among which are various types of lympholytic agents: glucocorticoids, alkylating compounds, cAMP, differentiation inductors, and ionizing radiation.

Thus, in addition to confirming the radiobiological idea that the main reason for the development of lymphopenia is interphase and reproductive lymphocyte death, the results we have obtained attest that the factor of slowed lymph transport also makes an important contribution to developing lymphopenia, resulting in development of a deficit in circulating lymphocytes in the lymph flow. The magnitude of this contribution can reach 30–45% depending on RD and elapsed time after irradiation.

If, along with the development of this process, we consider the migration of lymphoid cells to the wall of lymphangions and angions shows this to involve 10–15% of the lymphoid forms of cells, i.e., impairment of the specificity of lymphocyte "homing", then the interphase and reproductive death of lymphocytes, and thus this process, make a 40–45% contribution to the development of lymphopenia. The increase in the titer of autoantibodies, the morphological substrate for the synthesis of which is lymphocyte cells, the absence of severe hyperproteinemia in the transport media in the irradiated animal's body (after all the death of any cell forms should be accompanied by generation of protein, peptides, etc.) and the analysis of the changes over time in absolute numbers of lymphocytes, the subpopulations (and their active capacity to form rosettes with sheep erythrocytes and of erythrocytes and platelets all support this hypothesis.

The experimental group consisted of animals with normal blood profiles and body temperature, in which the homeostatic constant of central lymph did not exceed the limits of normal variability, which we had previously determined for five species of mammals since this was of fundamental importance to our study. Throughout the entire period of each experiment, ranging from 1 day to 2 years, the control and experimental animals were maintained under identical conditions of feeding, housing, and care, corresponding to livestock maintenance requirements and standards.

Mild, moderate, severe, and extremely severe acute radiation sickness, of the typical form as well as the gastrointestinal, toxics and cerebral syndromes were induced in the experimental groups of animals. The animals were irradiated in RUM-17, Puma, and Panorama devices. The exposure dose rate ranged from 3 A/kg to 29 A/kg. On the day preceding radiation exposure, and also on 15, 30, and 45 days postexposure, a lymphopenous anastomosis was created in the animals using the method described in Maliyev, Papov, 1989. Throughout the entire postirradiation period, samples of central lymph and peripheral blood were taken from the animals and used for measurement of cytochemical parameters.

The methods of immune depletion, affine immuno-lysopho-plasmasorption as well as direct extractions were used to extract the specific radiation (immunochemical)
Determinants (SRD) from the central lymph of animals with cerebral (SRD 1), toxic (SRD 2), gastrointestinal syndromes (SRD 3) and typical (mild-SRD 4/1, moderate SRD 4/2; severe SRD 4/3; and extremely severe SRD 4/4) forms of ARS. The specificity of the SRD was determined through the reactions immunosorbent assay (ELISA).

If analysis results were positive, blood was taken, serum separated out and IgG isolated (attention was focused on central lymph and peripheral blood). This allowed us to detect the presence and severity of radiation injury in the animals by identifying SRD-1; 2; 3 and 4 specific to (different levels of) radiation energy and also the radiation antibodies specific to each form of radiation sickness (ELISA-enzyme-linked immunosorbent assay method). Reactions were visually assessed, based on the difference between the color intensity of the reaction products for the experimental and control groups, as well as being measured with a scanning spectrophotometer with wave length of 492 nm. Visual assessment was performed within a 4 point scale (+++). The test was considered positive if the assessment was ++ or higher. When the reaction result was recorded spectrophotometrically, the coefficient of specificity (CS) was computed. A reaction was considered positive if the CS was 2.0 or more and negative when it was less than 2.0.

Immunochemical reactions were induced between the SRD preparations and anti-SRD in order to obtain a soluble immune complex. The following procedure was used: 4-5 µl of SRD antibody (precipitated fraction at 33% saturation of an ammonia sulfate solution of anti-SRD with a titer of 1:1000) was added to a 0.5 ml solution of SRD antibody specific to each form of radiation sickness (ELISA-enzyme-linked immunosorbent assay method). Reactions were visually assessed, based on the difference between the color intensity of the reaction products for the experimental and control groups, as well as being measured with a scanning spectrophotometer with wave length of 492 nm. Visual assessment was performed within a 4 point scale (+++). The test was considered positive if the assessment was ++ or higher. When the reaction result was recorded spectrophotometrically, the coefficient of specificity (CS) was computed. A reaction was considered positive if the CS was 2.0 or more and negative when it was less than 2.0.

The ELISA method prototype and the comparative analysis of SRD samples of various epitopes were based on Kuzin’s method (20), which was designed for identification of quinoid radiotoxins (45; 46). The comparative analysis of SRD samples of various epitopes was performed using a SR-20 (FRG) IR spectrometer using the method described in (Lekont, 1958; Peters et al., 1978).

Radiometric properties of SRD preparations were evaluated on the basis of capacity to induce radiobiological effects in the animals after they were administered parenterally. Lymphocytes of SRD 1 and SRD 4/4 (isolated from the lymph of animals irradiated at doses inducing cerebral and extremely severe ARS) were dissolved in an isotonic solution of NaCl. The dose of preparation administered was based on computation of the amount of SRD per unit volume of central lymph and absorbed dose of radiation. Symptoms of ARS induced with SRD 1 and SRD 4/4 were compared with similar symptoms induced by irradiation.

All experimentation procedures were in compliance with the Law and the International Guiding Principles for Biomedical Research Involving Animals as issues by the Council for the International Organizations of Medical sciences and EU Directives and EU recommendations. The studies were approved by the Animal Care and Use Committee for ethical animal research equivalent, at each institution.

The results of the studies were analyzed using ANOVA (STATISTICA 6.0 programme).

Results. Substances isolated from the central lymph of animals suffering from typical, gastrointestinal, toxic and cerebral forms of ARS have the appearance of light beige to light yellow powder, which adheres somewhat to glass, is soluble in water, aqueous solutions of NaCl, and alcohol, and reacts slightly to quinoid radiotoxins. Neutral aqueous solutions were used.

The components of SRD 3 and SRD 4/4 (Table 1) were isolated from the central lymph in the gastrointestinal and extremely severe forms of ARS. The increase of level of absorbed dose from the typical form of ARS to the gastrointestinal form is accompanied by a 6-8% growth in the protein and lipid components and a stable level of carbohydrates. The mineral residue decreases by 2%. The results of the chromatographic analysis revealed the relative homogeneity of the SRD preparations, and according to computations, the molecular mass of the isolated determinants were on the order of 200–250 kDa. This circumstance is responsible for SRD being transported through the lymphogenic route.

Table 1. Components of SRD 3 and SRD 4/4

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Intestinal SRD 3</th>
<th>Typical SRD 4/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Proteins</td>
<td>50.1 ± 0.09</td>
<td>56.2 ± 0.12</td>
</tr>
<tr>
<td>2. Lipids</td>
<td>38.2± 0.04</td>
<td>30.1± 0.09</td>
</tr>
<tr>
<td>3. Carbohydrates</td>
<td>10.2 ±0.03</td>
<td>10.1 ± 0.07</td>
</tr>
<tr>
<td>4. Mineral residue</td>
<td>1.3 ±0.04</td>
<td>3.4 ±0.17</td>
</tr>
</tbody>
</table>

The IR-spectra of the preparations showed both the general and the individual features of SRD. In particular, the IR-spectra of SRD 3 and SRD 4/4 suggest that both these determinants are compounds of identical type. This was confirmed by ELISA reactions, where we observed cross reactions between SRD 4.4 and antigens to SRD 3 and vice versa.

The results of immunochemical analysis of SRD 3 and SRD 4/4 suggest that their molecules consist of the identical stable heavy chain macromolecules, and relatively variable light chain molecules, which define the SRD 3 and SRD 4/4 epitopes. The high immunogenicity and specificity of the isolated SRD 1-4 preparations allowed using them as antigens for conducting ELISA analyses to diagnose radiation injuries of animals. This allowed us detecting "radiation" antigens (SRD 1-4) in the blood serum, urine, and organs of animals during the first 2 months after radiation exposure (Table 2) and the "radiation" antibodies to them over the subsequent 2 years (observation period). With regard to the chemical nature of isolated determinants of SRD 3 and SRD 4/4, it should be noted that they are conjugated proteins and their prosthetic group contains lipid and carbohydrate...
components. This type of conjugated protein is a component of the cell membranes and intracellular biomembranes of the nucleus, mitochondria, and microsomes and also is present in a free state (in physiological transport media). It has been established that an analogous type of conjugated protein participates in the structural, complex organization of myelin sheaths, nerves, chloroplasts, photoreceptor and electron transport systems, and the rods and cones of the retina (Berezov et al., 1982).

As for the mechanisms through which the conjugated proteins we have isolated from after radiation exposure, we have two working hypotheses:
- freeing of stored conjugated proteins of this class that were present earlier, followed by their modification;
- radiation-chemical and enzymatic processes responsible for new formation of lipo-glyco-proteins.

Existing data attest to the fact that various noncovalent forces, determined by the presence or absence of lipid components of the ionized group of atoms, participate in "radiation" glycoproteins that may be classified as lymphotoytic agents, since they are adducts triggering the induction of programmed death, the formation of conjugated proteins of this type.

In the series of experiments we studied the formation of SRD in central lymph and peripheral blood in animals suffering from different types of ARS. The results of these studies are presented in Table 2.

Table 2. Specific immunochemical reactions in different types of ARS

<table>
<thead>
<tr>
<th>Type of ARS</th>
<th>Visual and spectrophotometric assessment of ELISA (± and CS)</th>
<th>SRD1</th>
<th>SRD2</th>
<th>SRD3</th>
<th>SRD4/4</th>
<th>SRD4/3</th>
<th>SRD4/2</th>
<th>SRD4/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral</td>
<td>ppppp</td>
<td>2.8-3.3</td>
<td>2.7-3.0</td>
<td>2.6-2.9</td>
<td>2.2-2.9</td>
<td>2.1-2.2</td>
<td>2.0-2.1</td>
<td>1.8-2.0</td>
</tr>
<tr>
<td></td>
<td>ppppp</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Toxic</td>
<td>+ + + +</td>
<td>1.8-2.1</td>
<td>2.5-3.1</td>
<td>2.2-2.4</td>
<td>2.2-2.4</td>
<td>2.1-2.3</td>
<td>1.8-2.0</td>
<td>1.8-2.0</td>
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<tr>
<td></td>
<td>+ + + +</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>&lt;2.0</td>
<td>2.2-2.5</td>
<td>2.6-3.1</td>
<td>2.2-2.5</td>
<td>2.2-2.5</td>
<td>1.8-2.0</td>
<td>1.8-2.0</td>
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<td></td>
<td>+ + + +</td>
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<td>+</td>
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<tr>
<td>Typical:</td>
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<td></td>
</tr>
<tr>
<td>a) extremely severe</td>
<td>&lt;2.0</td>
<td>2.1-2.2</td>
<td>2.5-3.0</td>
<td>1.2-2.2</td>
<td>1.8-2.0</td>
<td>1.8-2.0</td>
<td></td>
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<tr>
<td></td>
<td>&lt;2.0</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b) severe</td>
<td>&lt;2.0</td>
<td>2.2-2.5</td>
<td>2.6-3.0</td>
<td>2.2-2.5</td>
<td>1.8-2.0</td>
<td>1.8-2.0</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>&lt;2.0</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c) moderate</td>
<td>&lt;2.0</td>
<td>2.0-2.1</td>
<td>12.0-2.1</td>
<td>2.5-2.6</td>
<td>2.0-2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;2.0</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d) mild</td>
<td>&lt;2.0</td>
<td>2.5-3.0</td>
<td>2.0-2.0</td>
<td>2.0-2.0</td>
<td>2.5-3.0</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>&lt;2.0</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Three to five hours after irradiation (Table 3) that induced a mild form of ARS, there was an increase in level of SRD4/1 in the lymph and blood (after 6–20 hours) of irradiated animals to the maximum level. SRD4/1 remained at that plateau for 5–20 hours. The antibody titer to SRD4/1 in lymphplasma and blood serum pre-irradiation was 1:80-1:100, attesting to the lack of SRD4/1 in the animals’ bodies. After 50–60 hours, the antibody titer to SRD4/1 increased and reached a maximum on day 25–45. The coefficient of specificity ranged from 2.0–2.5. In individual animals the antibody titer to SRD4/1 remained at a relatively high level until 60 days and on a moderate level – with CS of at least 2 – for up to 2 years (the duration of the observation period). It should be noted that, along with SRD4/1 and the antibodies to it, the presence of SRD4/2, SRD4/3, and SRD4/4 and the corresponding antibodies was detected in all animal species, but in significantly lower titers (CS = 1.5–1.8).

Results of reactions to the presence of quinoid radiotoxins (QRT) measured according to the method described in (Bond et al., 1971) were negative both in lymph and in blood.

Table 3. Time to develop maximum level of SRD as a function of type and severity of acute radiation sickness

<table>
<thead>
<tr>
<th>Type and severity of ARS; SRD</th>
<th>Time interval after irradiation (hours) to develop maximum SRD level, animal species and irradiation subject</th>
</tr>
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<tbody>
<tr>
<td>SRD</td>
<td>Table 3.</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Dogs</td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
</tr>
<tr>
<td>Typical mild, SRD 4/1</td>
<td>3–8</td>
</tr>
<tr>
<td>Moderate SRD 4/2</td>
<td>3–10</td>
</tr>
<tr>
<td>Severe SRD 4/3</td>
<td>3–24</td>
</tr>
<tr>
<td>Extremely severe, SRD 4/4</td>
<td>1–24</td>
</tr>
</tbody>
</table>
However, in cases of moderately severe ARC the formation of a positive level of QRT and maximum SRD in lymph preceded such levels in blood by an average of 2–5 hours and was observed in experimental animals, regardless of species, during the period 3–10 hours after irradiation. The reliability of the aniline reaction to QRT, as assessed on the basis of optical parameters of lymph samples studied, differed significantly from the reliability of results of ELISA analysis for the presence of SRD 4/1 (p<0.05 and p<0.001), and furthermore, after 24 hours, reactions to quinoid products became negative. Thus, along with theoretical indications, a whole series of experimental factors indicate that the reaction of SRD and anti-SRD binding is an immunochemical reaction between an antigen and an antibody. At the same time, the presence of QRT is based on a physicochemical reaction, which unambiguously argues that a specific immunochemical reaction underlies the mechanism through which the radiobiological effect is realized. It is not precluded, however, that the use of conjugates, in which a quinoid product serves as the hapten, could produce a positive result. The weak reaction to the quinoid product when the latter is measured in SRD preparations using A.M. Kuzin’s methods argues in favor of this.

The coefficient of specificity resulting from the ELISA test of SRD 4/1 in lymph was 2.0–2.1, while that for SRD 4/2 was 2.5–2.6. These results demonstrate that specific immunochemical reactions in animals with moderate levels of ARS generate less SRD 4/1 and more SRD 4/2, which is also demonstrated by the titer of antibodies relative to the determinant the level of which, like the level of corresponding antibody titers showed more individual to individual than species to species variation.

Thus, the blood serum of certain rabbits that showed an earlier reaction of active antibody development contained high titers of antibodies to SRD 4/1 and SRD 4/2, which continued to be observed for the entire 2-month observation period. The blood serum of cattle exhibited high antibody titers only to SRD 4/2 (from 2 weeks to 2 months) and some dogs, during the initial period, exhibited high antibody titers (CS=2.4) to SRD 4/1, and subsequently to SRD 4/2 (likewise persisting until month 2).

It should also be noted that animals whose blood and lymph contained high titers of antibodies to SRD4/2 and 4/1 after irradiation showed, as well as high antibody titers to these substances (1:2008–1:24488), did not exhibit either of these characteristics (antibody titers were 1:80–1:100) before irradiation. The leading determinant in the gastrointestinal syndrome of ARS, as revealed by the ELISA method, was SRD3 (CS=2.6–3.1). However, seropositive results were also obtained to the determinants characteristic of severe and extremely severe levels of ARS, i.e., SRD4/3 and SRD4/4 (CS ranged from 2.2–2.5). While in the typical form of ARS the results of the ELISA method for SRD 2 were seronegative, in the gastrointestinal ARS syndrome the CS for SRD2 was 2.2–2.5 which is characteristic of the toxic syndrome of ARS.

Irradiation of animals in doses inducing the toxic form of ARS led to the formation of SRD2 determinants with CS of 2.5–3.1, with seropositive results to the presence of SRD3, SRD4/4, and SRD4/3, but lower specificity (CS was 2.1–2.4).

In the cerebral ARS syndrome, the leading determinant was SRP 1, with seropositive results to the presence of SRD2, SRD3, SRD4/4 and SRD4/3. It should be pointed out that irradiation in the dose range evoking ARS from the extremely severe degree to the cerebral syndrome did not lead to formation of the determinants characteristic of the mild and moderate forms of ARS, i.e. SRD4/1 and 4/2 (Table 3).

In our investigation of migration of SRD4/4 using the method of extracorporeal diversion of lymph (EDL) in rabbits, dogs, and cattle suffering from extremely severe ARS we established that the level of SRD4/4 in blood after lymph diversion falls by an average of 30–50% (p<0.005), and the peak of maximum saturation is displaced to the right along the abscissa.

When we compared the time course of SRD4/4 formation in blood and lymph with the time course of lymph flow in the thoracic duct we noted a correlation between these processes. Thus, substantial shifts in lymph transport were noted 48–72 hours after irradiation and the maximum concentrations of SRD4/4 in lymph and blood were noted at 3–10 and 3–19 hours respectively. Thus the rate of SRD 4/4 input into blood through lymph flow during the first few hours after irradiation is very significant. As the lymphostatic process induced by radiation exposure evolves, there is a decrease in the level of SRD4/4 in blood.

In severe and extremely severe ARS, in addition to SRD 4/3 and 4/4 and the corresponding antibodies, we always found SRD4/1, SRD4/1 and their specific antibodies in the animal’s lymph and blood. However their coefficients of specificity ranged from 1.6–2.0. It should be specially noted that SRD3 was present in the lymph and blood of the animals that ultimately died (KOZ.O) and absent in those who survived (Table 3).

Thus, the high levels of CS along the “diagonal” in Table 3, which starts with a mild degree of ARS(minimal doses of radion were used in these experiments) and ends with the cerebral ARS syndrome attests to a immunochemical reaction specific to the radiobiological effect in the range of ARS forms studied.

These results show that the radiosensitivity of animals is determined by the nature of the specific immunochemical reaction to exposure to radiation. Thus, in animals irradiated in doses that are lethal to them, SRD1 2 and 3 predominate, but the absorbed of radiation is not important. If ELISA analysis shows a CS of more than 2.0 (for these determinants) the death of the irradiated animal is inevitable. Thus, the identical dose of radiation leads to the formation of specific determinants with different epitopes that determine the outcome of acute radiation sickness not only the bodies of animals of different species, but in individuals of the same species.

We performed experiments to study whether symptoms of acute radiation sickness could be induced
using SRD1 and SRD4/4 isolated from the lymph of animals suffering from the cerebral syndrome and extremely severe form of ARS. The determinants were administered intramuscularly; control animals received normal saline solution instead.

The radiobiological effects induced by SRD1 and SRD4/4 were compared to the analogous effects induced by irradiation. Then, on the basis of clinical and autopsy results, we determined the form and severity of the illness induced by the SRD.

Injection of the SRD1 preparation in 3 test doses caused the animals to die during the first day after injection. In these experimental animals, a period of extreme agitation was replaced by a deep coma, which ended in death. The results of the autopsy of their bodies showed the following: parenchyma organs filled with blood, punctuate bleeding on serous membranes, and cerebral tracts. Comparing the results obtained with those in the literary sources, we concluded that the SRD1 injection, when administered parenterally, induces in experimental animals the radiobiological effects characteristic of the cerebral ARS syndrome.

The clearest effects characteristic of radiation exposure occurred in animals, who received intramuscular injections of an SRD4/4 preparation, in three test doses. After 15 days had elapsed, all animals of this group had died on days 9, 12 and 16.

The clinical physiological symptoms were: short-term agitation for the first 2 hours after administration of the preparation accompanied by fits of arrhythmia decrease in appetite and increased peristalsis. Short-term leukocytosis gave way to a progressive increase in leukopenia, mainly attributable to decrease in the absolute number of lymphocytes, minimal levels of which were found between days 7 and 15 after injection. The amounts were 1.2–1.6 1000/µl and 0.4–0.5 thousand/µl in sheep and 1.8–2.5 thousand/µl and 0.5–0.7 thousand/µl in cattle. Recovery of absolute and relative levels of leukocytes and lymphocytes was observed in some animals between days 30 and 60.

Platelet levels exhibited thrombocytopenia accompanied by progressive erythrocytopenia (in animals of the third experimental group), which developed into anemia.

An extensive blood analysis of the peripheral blood of the cattle showed that the processes induced by the SRD4/4 injection and the processes occurring after irradiation were of the identical type (Kirshin et al., 1986; Bond et al., 1971).

Analysis of the development of the clinical reaction to SRD4/4 administration, which was assessed on the basis of body temperature, heart and respiration rate, established that all experimental animals showed reactions of the same type for all the tested doses and that sheep and horses were more sensitive to the administered preparation. Thus, in sheep in experimental group three, which received SRD 4/4 in the maximum dose tested, 1 or 2 days before death body temperature increased by 1.5–2°C, reaching 41.2°C. This was accompanied by severe tachycardia and tachypnea, which were measured at 105–106 and 70–80 per minute, respectively. When SRD4/4 was administered in the intermediate and maximum dose levels, sheep and cattle showed changes of the same type but of lesser degree. The majority of experimental animals had recovered between days 30–60 after injection of the preparation.

Autopsy on the animals that died showed changes characteristic of acute radiation sickness accompanied by marked haemorrhagia. Some sheep showed areas of epilation on the back and abdomen.

Comparison of the clinical picture and the changes revealed by autopsy and consideration of the time course of animal deaths induced by SRD with various epitopes and in various doses, led us to conclude that isolated determinants from central lymph of animals irradiated in a broad range of doses manifest marked radiomimetic effects characteristic of the symptoms of acute radiation sickness of one or another form.

Thus, the research performed suggests that when animals are irradiated in doses inducing development of cerebral (1), toxic (2), gastrointestinal (3) and typical (4) forms of acute radiation sickness, the radiobiological effects are of the nature of a specific immunochemical reaction, which takes the form of generation in the lymphoid system of a high molecular mass MN 200–250 kDa glycoprotein (SRD) with a specific epitope for each form of radiation injury (SRD 1; 2; 3; 4). Species and individual radiosensitivity of animals is determined by the individual's capacity to respond to radiation exposure by the formation of the dominant specific determinant of the corresponding epitope. In other words, the severity and final result of the radiobiological effect induced by the identical radiation dose in individuals of the same or different animal species depends on which of the glycoprotein epitopes forms in the body of the irradiated animal.

Despite the fact that the pathogenic mechanism underlying formation of the radiobiological effect has been considered in the literature since 1896, the immunochemical specificity of this mechanism has not been investigated until now.

The experimental confirmation of this hypothesis of the specificity of the immunochemical reactions underlying the radiobiological effects supplied by the present studies has provided us with the know how to create experimental samples of “antiradiation vaccines” and specific means of therapy and diagnosis of radiation injuries.

Experiments to determine optimal dose and administration times of an “antiradiation vaccine” – SAV (specific antiradiation vaccine) – in order to evaluate its radioprotective properties were performed on Wistar line rats aged 3–4 months with live weight of 180–200 kg, Chinchilla line rabbits, 11–13 months old and live weight of 3.5–3.7; and mixed breed dogs, 3 to 4 years old, with live weight of 6–6.5 kg. There were 20 experimental and 20 control animals in each study. The experiment was replicated three times. The exposure dose rate ranged from 3 A/kg to 29 A/kg. Five, 10, 15, 30, 60 and 90 days before irradiation, the animals received subcutaneous
injections of an antiradiation vaccine in doses of 5, 10, 15, or 20 µg/kg live mass. The control animals were injected with 1.5 ml of normal saline solution. The criterion for vaccine efficacy was survival of the animals 30 days after irradiation: for rats at a dose of 10.0 Gy, rabbits at a dose of 9.5 Gy, and dogs at dose of 6.5 Gy. The results are presented in Table 4.

Table 4. The determination of optimal dose and administration times of “antiradiation vaccine”

<table>
<thead>
<tr>
<th>No.</th>
<th>Group &amp; species</th>
<th>SAV dose (µg/kg)</th>
<th>N</th>
<th>Irrad. dose (Gy)</th>
<th>Number of days before irradiation SAV administered/ Percent animals surviving for 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>5</td>
<td>20</td>
<td>10.0</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>Rats</td>
<td>10</td>
<td>20</td>
<td>10.0</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>Rats</td>
<td>15</td>
<td>20</td>
<td>10.0</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>Rats</td>
<td>20</td>
<td>20</td>
<td>10.0</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Control (radiation only)</td>
<td>--</td>
<td>20</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Rabbits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>5</td>
<td>20</td>
<td>9.5</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>Rabbits</td>
<td>10</td>
<td>20</td>
<td>9.5</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>Rabbits</td>
<td>15</td>
<td>20</td>
<td>9.5</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>Rabbits</td>
<td>20</td>
<td>20</td>
<td>9.5</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Control (radiation only)</td>
<td>--</td>
<td>20</td>
<td>9.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>5</td>
<td>20</td>
<td>6.5</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>Dogs</td>
<td>10</td>
<td>60</td>
<td>6.5</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
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<td>Dogs</td>
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<td>20</td>
<td>6.5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Control (radiation only)</td>
<td>--</td>
<td>20</td>
<td>6.5</td>
<td>0</td>
</tr>
</tbody>
</table>

It follows from Table 4 that the maximum radioprotective effect of SAV occurs at doses of 10–15 µg/kg and administration times 10-60 days before exposure to lethal doses of radiation, ensuring survival rates of 95–100% in the experimental animals. It should be noted that doses of 5 or 20 µg/kg administered 10–60 days before irradiation also had a high radioprotective effect (70–90%), and administration of SAV 5 and 90 days before irradiation ensured survival rates of 40 to 90%. Fatality rate was 100% in the control conditions.

Extensive information about the efficacy of the “antiradiation vaccine” is provided in Table 5.

Table 5. The effect of “antiradiation vaccine” (after exposure to lethal doses of up to 10 Gy)

<table>
<thead>
<tr>
<th>No.</th>
<th>Animals:</th>
<th>Group</th>
<th>Exposure Dose, Gy</th>
<th>Survival rate in % after exposure period of (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 days</td>
</tr>
<tr>
<td>1</td>
<td>Mice</td>
<td>Experimental</td>
<td>7.0</td>
<td>85-100</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>Control</td>
<td>7.0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Rabbits</td>
<td>Experimental</td>
<td>9.5</td>
<td>80-100</td>
</tr>
<tr>
<td></td>
<td>Rabbits</td>
<td>Control</td>
<td>9.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Dogs</td>
<td>Experimental</td>
<td>6.5</td>
<td>95-100</td>
</tr>
<tr>
<td></td>
<td>Dogs</td>
<td>Control</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Pigs</td>
<td>Experimental</td>
<td>7.5</td>
<td>95-100</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>Control</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Sheep</td>
<td>Experimental</td>
<td>6.5</td>
<td>80-100</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>Control</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Horses</td>
<td>Experimental</td>
<td>6.5</td>
<td>75-100</td>
</tr>
<tr>
<td></td>
<td>Horses</td>
<td>Control</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Cattle</td>
<td>Experimental</td>
<td>9.2</td>
<td>95-100</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Control</td>
<td>9.2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Rats</td>
<td>Experimental</td>
<td>8.5</td>
<td>90-100</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>Control</td>
<td>8.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion. The results obtained may be applied extensively to technologies in radiobiology and the production of drugs for use during space flights of humans and animals under conditions of elevated radiation risk and travel to areas close to high radiation sources, to increase the immunogenic properties of vaccines (foot and mouth disease, paratyphoid, and anthrax) and sera used for diagnosing parasitic diseases.
unambiguously identify radiation injury.

Using the radiation antigen, which makes it possible to

on the "ELISA-based diagnosis of radiation injuries"

the "radiation" antigen; possession of "know-how" to isolate

isolating the "radiation" antigen; a method of using the

a follows: a substance - a "radiation" antigen; a method of using the

research have been used to develop material for patenting

lipopolisaccharides, proteins, lipids). The results of the

membranes) and molecules (glicoproteins, lymphoid tissue to

formation of radiation induced toxins in radiosensitive

Cell and red cell progenitors.

Radiation toxin SRD-4 (hematotoxin) induces

mimicked gastrointestinal acute radiation syndrome.

hematochezia were important clinical indicators of the

crypt / villi necrosis. Severe diarrhea and melena /

apoptosis of intestinal lining epithelial cells, including

network of the gastrointestinal system, as well as

effects and damage to the vascular and lymphatic vessel

network of the gastrointestinal system, as well as

as apoptosis of intestinal lining epithelial cells, including
crypt / villi necrosis. Severe diarrhea and melena /

radiotherapy for cancer; to

decrease side effects of radiation therapy for cancer; to

and humans from the consequences of radiation exposure

"antiradiation vaccines" to prevent and protect animals

major uses of radiation antigen are: manufacture of

processes: breaking of the chemical bonds of

lymph fluid.

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