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RADIOSTRONTIUM-INDUCED ONCOGENESIS AND THE ROLE OF IMMUNOSUPPRESSION

II. Influence of ^{90}Sr dose, adult thymectomy and antilymphocyteglobulin treatment on the development of lympho-reticular and extraskeletal, neoplastic lesions in CBA mice

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Abstract

The significance of depressed immune function for the development and progression of tumours induced by ^{90}Sr (mainly osteosarcomas and malignant lymphomas) was investigated in a series of experiments by comparing the tumour responses in normal mice with those in immunocompromised mice. The present paper (part II) reports on lympho-reticular (LR) and extraskeletal neoplastic lesions in male CBA/SU mice after exposure to different single doses of ^{90}Sr with or without additional immunosuppression by adult thymectomy (ATx) and/or prolonged antilymphocyteglobulin (ALG) treatment. Neoplastic lesions in bone were reported in part I (1). The status of the animal's immune system and responsive ability were examined in parallel experiments. The tumour yields were analysed in relation to the dosage of ^{90}Sr and the immunosuppressive treatments employed. Although the incidences and latency times of induced tumours were clearly dose-dependent, they were never significantly influenced by ATx/ALG treatments. Thus, no substantial support was gained for the theory that the immune system plays a controlling or modifying role in ^{90}Sr carcinogenesis. The results, which are in agreement with the bone tumour responses, suggest that ^{90}Sr induced tumours either do not express the antigens necessary for immune rejection or that the decline in immune responsiveness induced by ATx/ALG was of little consequence for tumour development and spread. The pathogenesis of ^{90}Sr induced malignant lymphomas (MLs) and their immunophenotypes are discussed.

Key words: Radiation carcinogenesis, strontium-90, immunosuppression, lympho-reticular tumours, extraskeletal tumours.

The bone-seeking, β-emitting radionuclide ^{90}Sr (half-life 28 years) is a potent carcinogen in a variety of mammalian species. Malignant bone tumours have been reported in ^{90}Sr exposed dogs (2–5), monkeys (6), pigs (7), rabbits (8), rats (9–11) and mice (1, 12–15). Neoplastic haematopoietic tissue lesions have been produced in dogs (16), pigs (17), rats (11), and mice (18–20). Other ^{90}Sr related neoplastic lesions observed in mice include carcinomas of the oral mucosa (21) and the external ear (22), and pituitary tumours (23).

Much debate has long centered around the hypothesis that various phases of radiation induced tumour development and spread are a result of a failure by the immune system to respond adequately. Still, no firm consensus has been reached, and more investigations are needed—especially with regard to long-term immunosuppression and life-span effects. In the present investigation the tumour yields in mice induced by injection of various single doses of ^{90}Sr were compared with the tumour yields in mice simultaneously exposed to unspecific immunosuppression by adult thymectomy (ATx) and/or long-term treatment with antilymphocyteglobulin (ALG).

The hypothesis that a failure in normal immune function might play a potentially decisive role in the process of radiogenic tumour formation, progression and dissemination was derived from diverse suggestive observations. Of particular interest are those related to treatment with radioactive strontium, namely a) the dose dependency of neoplastic responses (14, 19–22) as well as immunologic

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suppression (24), b) the dose-determined relative representation of various osteosarcoma subtypes, i.e. predominance of well differentiated osteoblastic osteosarcomas at high dose levels and less well differentiated, predictedly less immunogenic subtypes at lower dose levels (14, 25), c) the selective depletion of natural killer (NK) cells (unpublished observations, 26), which are thought to have a relevant function in protection against tumour development and/or dissemination of tumours (27, 28), d) the reduced osteosarcoma incidence and prolonged induction time following immune stimulation by BCG (29), and e) the reduced acceptance of osteosarcoma transplants following preimmunization with osteosarcoma cells (25).

Haematopoietic tissue tumours and extraskeletal tumours are reported in this paper (part II), and the overall tumour responses, including the osteosarcomas reported previously (1), are evaluated with reference to ^{90}Sr activity and the immunosuppressive treatment used. To allow interpretation of these experiments, parallel sets of similarly treated mouse groups, coded B–F, were used to estimate their respective immunologic responsive capacity. These studies included allogenic skin graft rejection (30), RES-phagocytic function and *in vitro* responsiveness of mononuclear cells to the mitogenes PHA, Con-A and LPS (unpublished), enumeration of peripheral blood white cells, specifically T-cells (31, 32), and assessment of natural killer (NK) cell activity (unpublished).

Taken together these studies demonstrated that ATx/ALG treatment constitutes an efficient method to increase immunosuppression in ^{90}Sr exposed mice. The profound and protracted extinction of the cell-mediated immune system by ATx/ALG treatment was particularly well evidenced by the acceptance of allogenic skin grafts (30).

Material and Methods

Experimental design. The experimental plan and procedures are described in full detail in part I of this report (1). Briefly, four series of male CBA/SU mice were injected intraperitoneally with different single doses of $^{90}Sr(NO_3)_2$ (*Series 1;* 29.6, *Series 2;* 14.8, *Series 3;* 7.4 and *Series 4;* 1.85 kBq ^{90}Sr/g body weight respectively) at the age of 75±3 days. In addition, each series contained subgroups that were immunosuppressed by adult thymectomy (ATx) and/or prolonged antilymphocyteglobulin (ALG) treatment (interval 172–305 days after ^{90}Sr) in combinations as depicted in the tables and figures.

Pathology. The animals were allowed to live until they were moribund, and then euthanized with ether. Complete autopsies were performed, including both gross and microscopic evaluations. Tissues were fixed in Stieve's solution for 12 h, and cross sections for microscopy were prepared as described previously (1). Sections were stained by the van Gieson method, with hematoxylin and eosin (HE) and with methyl green-pyronin (MGP) or toluidine blue. The histologic typing of tumours was done in accordance with the system recommended by the European Late Effects Project Group (EULEP) Committee on Pathology Standardization (33).

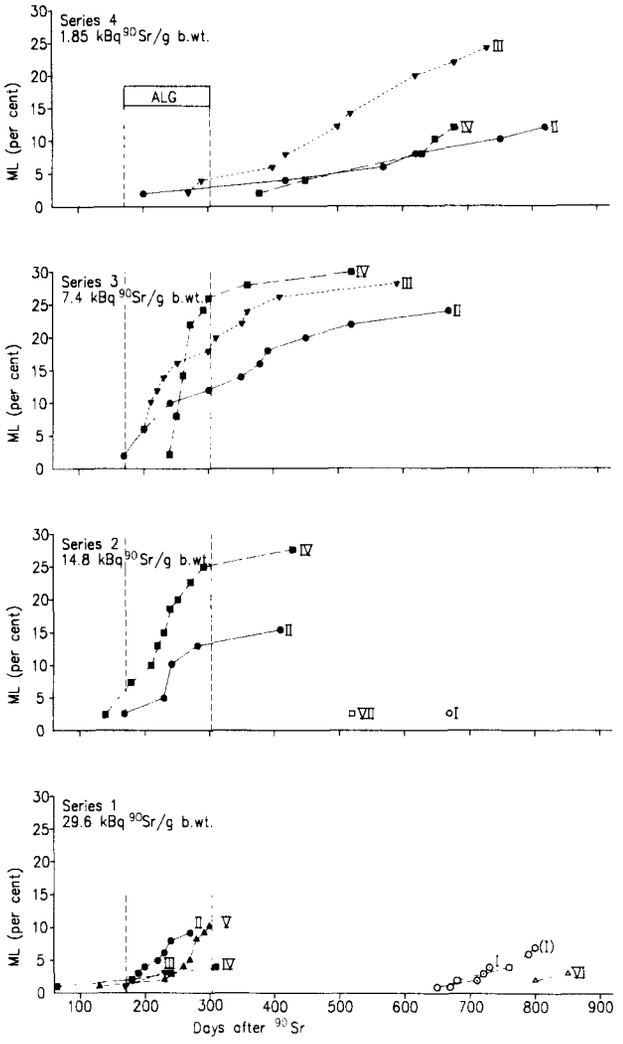

Figure. Cumulative incidence (%) of malignant lymphomas (ML) in Series 1, 2, 3, and 4, registered at 10-day intervals. ALG =weekly injection of antilymphocyteglobulin, days 172–305. (I)=untreated control (○—○), I=untreated cagemate control (⊙—⊙), II=^{90}Sr (●—●), III=^{90}Sr+ATx (▼---▼), IV=^{90}Sr+ATx+ALG (■---■), V=^{90}Sr+ALG (▲--▲), VI=ALG (△--△), VII=ATx+ALG (□---□).

Statistics. Neoplastic responses were expressed as the crude number and percentage of various tumour types found in ech group of mice. When evaluating the probability of developing malignant lymphoma, appropriate corrections were made for competing mortality (Reviews and comments: 34–37).

Results

All neoplastic lesions diagnosed in each group, with the exception of osteosarcomas (1), are presented in tabulated form according to their histologic type or subtype.

Lympho-reticular (LR) neoplasms

Malignant lymphomas (MLs) were the prevailing tumours and appeared earliest after radiation exposure. The ML incidence accumulated over time is illustrated for each dose-series in Figure, and a comparison between crude and actuarial data is presented in Table 2. Tumours of histiocytic and mast cell origin were also observed; however, they were infrequent and generally appeared late (Table 1).

Gross morphology of malignant lymphomas. When examined in a generalized stage of development, the MLs exhibited a more or less pronounced invasion of several organs. Still, one of three manifestation forms, suggesting a thymic, bone marrow or visceral site of origin, was almost always discernible and utilized for subclassification (Table 1), if not contradicted by the microscopic picture. The criteria used were as follows.

Thymic form: a) predominant gross lesion in thymus; b) limited or absent invasion of the spinal bone marrow, no invasion of the surrounding tissues; c) visceral organs may be affected but never very severely. *Bone marrow form:* a) massive proliferation in spinal bone marrow with or without heavy overgrowth to adjacent skeletal muscles; b) no invasion in thymus or discrete invasion in the case of coexistent gross lesion in the nearby mediastinal lymph nodes; c) visceral organs and lymph nodes sometimes affected but then only of subordinate magnitude. *Visceral form:* a) predominant gross lesion in visceral organs; b) invasion absent or restricted in the spinal bone marrow, no invasion of surrounding tissues; c) no invasion of thymus or discrete invasion in the case of coexistent gross lesion in the nearby mediastinal lymph nodes. *Advanced generalized form:* Primary growth site dubious owing to pronounced proliferation in lymph nodes and visceral organs as well as in thymus and/or bone marrow.

Microscopy of malignant lymphomas. The MLs varied considerably in their histologic appearance, depending on the representation and distribution of constituent lymphoid and histiocytic cells as well as on the type of cell arrangement (e.g. *cohesiveness*), cell morphology and degree of differentiaton. The majority of the MLs, however, were characterized by an abundance of diffusely growing, relatively uniform lymphoblastic cells. The nuclei, with finely dispersed chromatin, were mostly roundish and rarely invaginated or convoluted.

Heterogeneity among the lymphoid cells, manifested by interspersed populations of cells with poorly differentiated lymphocytic or plasmacytoid appearance, occurred occasionally and motivated the use of the subclassification presented in Table 1 (modified from EULEP). In 4 cases the presumptive diagnosis *immunoblastic lymphoma* was used. These morphologically separable tumours contained large, monomorphous cells with finely dispersed, delicate chromatin and occasionally vacuolated nuclei, often with one or two prominent nucleoli. In addition, in terms of their cytoplasmic appearance, some cells resembled immature plasma cells.

The diagnosis *histiocytic sarcoma* was here applied to tumours considered to be of true histiocytic origin (i.e., non-lymphoid phagocytic cells). These tumours typically developed late in life, originated in lymph nodes and metastasized occasionally to spleen or liver but never to the bone marrow. They were made up of a solid mass of polygonal and elongated cells with large, vesicular nuclei, which were usually roundish but could also be indented, reniform or even lobulated. Prominent nucleoli were also characteristically found as was the abundant, variably staining and often granular cytoplasma. In contrast to the MLs, the histiocytic sarcomas were not enhanced in the irradiated groups as compared with the non-irradiated ones, nor did they develop sooner (702–807 days vs. 635–856 days after start of experiment).

Mast cell tumours. Among the unirradiated mice, 8 (2.0%) were observed with mast cell tumours between 299 and 900 days after start of the experiment. In 6 of these cases growth was confined to the bone marrow and involved multiple compartments, e.g. femora and vertebrae. In the other two cases the tumours were localized in abdominal lymph nodes. Among the radiation-exposed mice three were observed with mast cell tumours (0.4%), 303 to 659 days after start of the experiment, all of which engaged multiple bones.

The histologic picture was that of a packed proliferation of large, polygonal to oval cells with abundant cytoplasma and a centrally located, round nucleus. The toluidine blue-positive cytoplasmic granules, characteristic of mast cells, were always present though they were occasionally scanty.

Other neoplasms

Tumours observed in organ systems other than the skeleton and the LR-tissues are presented in Table 3. The majority of these tumours obviously represent age-related phenomena unaffected by the treatments employed.

Hepatocellular and bronchiolo-alveolar tumours were the most prevalent of the spontaneously occurring neoplastic lesions. These tumours, however, did not develop until late in life and gradually converted into malignant form. Accordingly they were rare in mice exposed to severely life-shortening activities of ^{90}Sr, and increased gradually as the dose declined ultimately approaching the values of untreated controls. ^{90}Sr did not interfere in the genesis of these tumours, nor did ATx or ALG treatments cause such interference.

In Series 1, the frequency of *squamous cell carcinomas* in the nasal and oral cavities ranged between 15 and 20% in ^{90}Sr exposed groups as compared with zero to 1% in non-exposed groups. In addition, some squamous cell carcinomas and sebaceous cell carcinomas were observed in the deep bony portion of the aural duct of some ^{90}Sr exposed mice.

Table 1

Lymphoreticular (LR) tumours in Series 1, 2, 3 and 4: light microscopic classification, incidence and survival time (Mean ± SE) in male CBA mice treated as indicated in the table by thymectomy (ATx) at 60 days of age, intraperitoneal injection of ^{90}Sr at 75 days of age (=day 0) and by weekly injections of antilymphocyteglobulin (ALG) on days 172–305 after ^{90}Sr

Series 1 (29.6 kBq ^{90}Sr/g body weight)

Group code	A (I)	AI	AII	AIII	AIV	AV	AVI
Treatments	Contr. separated	Contr. cage mate	^{90}Sr	^{90}Sr ATx	^{90}Sr ATx ALG	^{90}Sr ALG	ALG
No. of mice	98	100	99	100	99	97	100
Malignant lymphoma (ML)							
Lymphocytic/lymphobl.		1	1		1	2	1
Lymphoblastic	3	2	5	3	3	7	1
Lymphobl./plasmacytoid	2	1	1			1	
Immunoblastic			2				1
Lymphoid/histiocytic[1]	2						
Total	7	4	9	3	4	10	3
ML manifestation forms[2]							
Thymic	0	0	0	–	–	2	0
Bone marrow	0	0	6	3	4	5	0
Visceral	5	4	0	0	0	3	1
Advanced generalized	2	0	3[3]	0	0	0	2[4]
Survival with ML, days (range)	745±21 (665–797)	698±18 (643–724)	216±10 (182–269)	206±19 (168–226)	195±51 (63–306)	254±15 (130–298)	636±187 (263–851)
Histiocytic sarcoma[5]		2					1
Mast cell leukaemia	3	1*	1*				2
Total	3	3	1	0	0	0	3
Total No. of LR tumours	10	7	10	3	4	10	6
Survival with LR tumour days (range)	738±25 (574–846)	658±62 (299–781)	224±13 (182–303)	206±19 (168–226)	195±51 (63–306)	254±15 (130–298)	735±99 (263–900)

Series 2 (14.8 kBq ^{90}Sr/g body weight)

Group code	AI	AII	AIV	AVII
Treatments	Control	^{90}Sr	^{90}Sr ATx ALG	ATx ALG
No. of mice	37	39	40	40
Malignant lymphoma (ML)				
Lymphocytic/lymphobl.		2	2	1
Lymphoblastic		4	9	
Lymphobl./plasmacytoid	1			
Total	1	6	11	1
ML manifestation forms[2]				
Thymic	0	0	–	–
Bone marrow	0	6	10	0
Visceral	1	0	0	1
Advanced generalized	0	0	1	0
Survival with ML, days (range)	664	256±33 (163–401)	236±23 (134–423)	517
Mast cell leukaemia	0	0	0	2
Total No. of LR tumours	1	6	11	3
Survival with LR tumour days (range)	664	256±33 (163–401)	236±23 (134–423)	740±113 (517–885)

(Table 1 cont.)

Series 3 (7.4 kBq ^{90}Sr/g body weight)

Group code	AI	AII	AIII	AIV
Treatments	Control	^{90}Sr	^{90}Sr ATx	^{90}Sr ATx ALG
No. of mice	30	50	50	50
Malignant lymphoma (ML)				
Lymphocytic/lymphobl.			1	
Lymphoblastic		10	10	12
Lymphobl./plasmacytoid				1
Plasmacytic			1	
Immunoblastic			1	
Lymphoid/histiocytic[1]		1		
Unclassified		1	1	2
Total	0	12	14	15
ML manifestation forms[2]				
Thymic	0	0	–	–
Bone marrow	0	7	9	10
Visceral	0	2	2	2
Advanced generalized	0	3	3	3
Survival with ML, days (range)		338±43 (166–666)	285±30 (193–588)	283±18 (238–514)
Histiocytic sarcoma[5]	2		1	
Mast cell leukaemia		1	1	
Total	2	1	2	0
Total No. of LR tumours	2	13	16	15
Survival with LR tumour days (range)	772±36 (736–807)	363±47 (166–666)	336±44 (193–792)	283±18 (238–514)

Series 4 (1.85 kBq ^{90}Sr/g body weight)

Group code	AI	AII	AIII	AIV
Treatments	Control	^{90}Sr	^{90}Sr ATx	^{90}Sr ATx ALG
No. of mice	30	50	50	50
Malignant lymphoma (ML)				
Lymphocytic/lymphobl.		1	2	1
Lymphoblastic		3	5	3
Lymphobl./plasmacytoid		1	1	
Plasmacytic		1		
Lymphoid/histiocytic[1]			1	
Unclassified			3	2
Total	0	6	12	6
ML manifestation forms[2]				
Thymic	0	0	–	–
Bone marrow	0	2	7	2
Visceral	0	1	2	2
Advanced generalized	0	3	3	2
Survival with ML, days (range)		559±93 (197–820)	490±41 (266–730)	565±50 (373–672)
Histiocytic sarcoma[5]	2	2	0	1
Total No. of LR tumours	2	8	12	7
Survival with LR tumour days (range)	772±36 (736–807)	606±77 (197–856)	510±43 (266–730)	586±47 (373–711)

[1] Congruent with reticulum cell sarcoma type B of Dunn (38).
[2] Manifestation form denotes the presumed site of primary growth.
[3] All with gross thymic lesion.
[4] One with gross thymic lesion.
[5] Congruent with reticulum cell sarcoma type A of Dunn (38).
* Localized.

Table 2

Comparison between crude incidence of mice with malignant lymphoma and corresponding actuarial appearance ± SE, i.e. probability of an animal developing a tumour

Series, group	Crude % [a]	Crude % [b]	Actuarial appearance[c]	Mean time of actuarial appearance of tumours (days)
1 A II	9	9	0.143±0.047	243±16
A III	3	3	0.032±0.018[d]	208±26
A IV	4	4	0.077±0.039	238±50
A V	10	10	0.172±0.056[d]	270±14
2 A II	15	15	0.183±0.069	271±36
A IV	27	27	0.313±0.080	250±27
3 A II	24	22	0.249±0.065	427±59
A III	28	28	0.321±0.076	316±38
A IV	30	30	0.303±0.064	285±19
4 A II	12	8	0.103±0.049[e]	728±69[e]
A III	24	24	0.332±0.082[e]	540±43[e]
A IV	12	12	0.161±0.060	582±47

[a] Incidence when all mice had lived their full life span.
[b] Incidence when 10 mice were still alive.
[c] Actuarial incidence based on tumour appearance up to the level of 10 surviving mice.
[d] A III vs. A V: $P<0.05$.
[e] A III vs. A II: $P<0.05$.

Combined treatment with ATx/ALG had no apparent influence on the development of squamous cell carcinomas.

Among the remaining tumours in Table 3 the esthesioneuroepitheliomas of the olfactory mucosa were the only ones pointing to a causal relation with ^{90}Sr.

Discussion

The possible significance of immune unresponsiveness in radiogenic cancer has proved difficult to evaluate experimentally, not least because of the lack of suitable experimental models. Chemical immunosuppressors are generally too toxic for prolonged use and may even be carcinogenic in themselves. The effects of monoclonal antibody-based selective depletion of lymphoid cell populations are of limited duration and the treatment is costly. A combination of ATx and ALG treatments turned out to be the best choice available and notably fulfilled the requirement of not shortening the animals' life span. The present experimental set-up, using ^{90}Sr exposed and immunocompromised mice, was consequently designed with the primary objective of elucidating the biological role of impaired immune function in radiation oncogenesis. It was also considered important to extend and confirm knowledge about the origin and pathogenesis of induced lymphomas.

The first and most obvious finding in this experiment was the induction of malignant lymphomas (ML) by ^{90}Sr. The pathology, latency, and rate of occurrence of the tumours at various dose levels were in good agreement with previous reports in the same mouse strain (18–20). Since the ML rates were influenced by deaths due to other causes, actuarial ML incidence and latency were determined in order to allow adequate comparison of data between mouse groups.

Among the unirradiated control mice, 4.5% (12/267) developed ML at senescence, between 643–797 days after starting the experiment. The corresponding figures in ATx and/or ALG treated mice were 2.8% (4/140) and 263–851 days. All these tumours had the 'visceral' manifestation form, if not advanced generalized, which clearly contrasts to the 'bone marrow' and 'thymic' forms seen in ^{90}Sr exposed mice. Thus the spontaneous MLs differed from the ^{90}Sr induced MLs in terms of origin and time of appearance, but not in terms of histology.

The Figure shows that the onset of ML formation in the group given the highest activity of ^{90}Sr started approximately 500 days earlier than in unirradiated controls and soon ceased to increase at a low cumulative level. This relatively low total can be attributed primarily to the effects of an irradiation overdose on the haematopoietic tissue and by the early appearance of osteosarcomas in this dose series, as evidenced histologically. In ^{90}Sr treated mice the ML incidence varied with the activity, with a maximum of 24% after 7.4 kBq ^{90}Sr/g bwt. The time elapsing before tumour appearance in all dose series was notably in the narrow range between 163 and 197 days and terminated as a function of the dose influence on survival.

Immunosuppression of ^{90}Sr exposed mice by ATx and/or ALG treatment did not significantly change the crude ML response in any systematic way or specific

Table 3

Extraskeletal, neoplastic lesions, not including lympho-reticular tumours, in male CBA mice treated as indicated in the table by thymectomy (ATx) at 60 days of age, intraperitoneal injection of ^{90}Sr at 75 days of age (=day 0) and by weekly injections of antilymphocyteglobulin (ALG) on days 172–305 after ^{90}Sr

Series 1 (29.6 kBq ^{90}Sr/g body weight)

Group code	A(I)	AI	AII	AIII	AIV	AV	AVI
Treatments	Contr. separated	Contr. cage-mate	^{90}Sr – –	^{90}Sr ATx –	^{90}Sr ATx ALG	^{90}Sr – ALG	– – ALG
No. of mice	98	100	99	100	99	97	100
Integumentary system							
Squamous cell carcinoma, aural duct		1	1		2	1	
Sebaceous gl. carcinoma, aural duct			2	2			
Ophthalmic system							
Harderian gl. adenoma	4	3	2		3		1
Harderian adenocarcinoma	1		1	1			1
Alimentary system							
Salivary gl. carcinoma	1						
Squamous cell carcinoma, oral cavity		1	12	14	14	17	
stomach	1						
Polypous adenoma, small intestine	1						
Mesothelioma, abdominal							1
Pancreatic exocrine carcinoma	1	1					
Hepatocellular adenoma	49	51	3	4	1	2	58
Hepatocellular carcinoma	18[3]	17[2]					14[3]
Cholangiocarcinoma	1						
Pleomorphic sarcoma, abdominal cavity		1					
Respiratory system							
Squamous cell carcinoma, nasal cavity			3	1	3	3	
Bronchiolo-alveolar tumour	17	11	1	1	4	3	11
Vascular system							
Haemangioma, spleen	2						
Haemangioma, testis					1		
Angiosarcoma, liver		1					
Reproductive system							
Leydig cell tumour, testis		1					
Endocrine system							
Adrenocortical adenoma		1			1		
Pituitary adenoma		1					
Pituitary carcinoma		1					
Nervous system							
Esthesioneuroepithelioma, olfactory mucosa			1	6	1		
Undefined origin							
Granular cell tumour, thoracic wall							1
Fibrosarcoma, abdominal					1		
Total	96	91	26	29	31	26	87

direction. A loss of immunocompetent cells could be expected to facilitate the progression of induced immunogenic, malignant cells. On the other hand, a reduction in the number of cells at risk for transformation could also counterbalance this effect. However, when turning to the actuarial data (Table 2) it can be seen that the immunosuppressive treatments tended to promote tumour development and shorten latency in Series 2, 3, and 4, but not in Series 1. These suggestive results obviously lack the weight necessary for making conclusive interpretations but can hardly be dismissed until further investigated. The trend breaking responses in Series 1 probably again reflect the status of the target tissues, i.e. the preconditions for neoplastic transformation and progression, as created by

(Table 3 cont.)

Series 2 (14.8 kBq ^{90}Sr/g body weight)

Group code	AI	AII	AIV	AVII
Treatments	Control	^{90}Sr	^{90}Sr ATx ALG	ATx ALG
No. of mice	37	39	40	40
Ophthalmic system				
Harderian gl. adenoma		1 (3)[a]	1 (3)	1 (3)
Alimentary system				
Squamous cell carcinoma, stomach	1 (3)			1 (3)
Mesothelioma, abdominal	1 (3)			1 (3)
Hepatocellular adenoma	18 (49)	13 (33)	14 (35)	13 (33)
Hepatocellular carcinoma	10[4] (27)	3 (8)	3 (8)	7[2] (18)
Respiratory system				
Fibrosarcoma, nasal cavity	1 (3)			
Bronchiolo-alveolar tumour	4 (11)		4 (10)	2 (5)
Vascular system				
Haemangioma, spleen	1 (3)			
Endocrine system				
Adrenocortical adenoma	1 (3)			
Phaeochromocytoma	1 (3)			
Total	38	17	22	25

Series 3 (7.4 kBq ^{90}Sr/g body weight)

Group code	AI	AII	AIII	AIV
Treatments	Control	^{90}Sr	^{90}Sr ATx	^{90}Sr ATx ALG
No. of mice	30	50	50	50
Integumentary system				
Fibrosarcoma, skin	1 (3)[a]			1 (2)
Opthalmic system				
Harderian gl. adenoma	2 (7)	2 (4)	2 (4)	2 (4)
Alimentary system				
Hepatocellular adenoma	16 (53)	17 (34)	21 (42)	20 (40)
Hepatocellular carcinoma	6 (20)	5[2] (10)	2 (4)	3 (6)
Respiratory system				
Squamous cell carcinoma, nasal cavity		1 (2)		3 (6)
Bronchiolo-alveolar tumour	4 (13)	5 (10)	5[2] (10)	5 (10)
Vascular system				
Angiosarcoma, lymph node		1 (2)		
Endocrine system				
Pituitary adenoma		1 (2)	1 (2)	1 (2)
Pituitary carcinoma				1 (2)
Nervous system				
Esthesioneuroepithelioma, olfactory mucosa				1 (2)
Skeletal muscles				
Rhabdomyosarcoma				1 (2)
Total	29	32	31	38

the balance between irradiation and immunosuppressive treatments on the one hand and regenerative potential on the other.

To improve the subclassification of MLs, efforts were made to combine immuno-phenotypic characteristics with the morphologic criteria. The preliminary results with immuno-peroxidase techniques to detect immunoglobulines, Thy-1, Lyt-1, and H-2 I-A antigens (Sera-Lab) in Stieve-fixed tumours revealed that at least some of the 'marrow manifestation form' of lymphomas were derived from the B cell lineage, while those with a 'thymic manifestation form' were derived from the T cell lineage. A detailed report on these observations will be included in a broad retrospective study when more reagents have been tested and the technique refined.

From the histopathology it was evident that ^{90}Sr induced MLs in the majority of cases were initiated in the spinal bone marrow, where the primary growth was ob-

(Table 3 cont.)

Series 4 (1.85 kBq ^{90}Sr/g body weight)

Group code	AI	AII	AIII	AIV
Treatments	Control	^{90}Sr	^{90}Sr ATx	^{90}Sr ATx ALG
No. of mice	30	50	50	50
Integumentary system				
Sebaceous gl. carcinoma				1 (2)[a]
Fibrosarcoma	1 (3)			
Opthalmic system				
Harderian gl. adenoma	2 (7)	2 (4)		2 (4)
Alimentary system				
Squamous cell carcinoma, stomach			1[1] (2)	
Mesothelioma, abdominal		1 (2)		
Hepatocellular adenoma	16 (53)	22 (44)	18 (36)	23 (46)
Hepatocellular carcinoma	6 (20)	1 (2)	11[2] (22)	13[5] (26)
Respiratory system				
Bronchiolo-alveolar tumour	4 (13)	2 (4)	7 (14)	5[1] (10)
Vascular system				
Angiosarcoma, liver			1 (2)	
Endocrine system				
Adrenocortical adenoma			1 (2)	1 (2)
Pituitary adenoma		1 (2)		
Total	29	29	39	45

[a] Number of animals with tumour (percentage).
[1,2,3,4,5] Figures indicate the number of mice with metastasis.

served. The clear preference for the thoracic region is another interesting observation. The explanation for this is probably a complex one, but obviously includes the exposure conditions there. The tumours with a thymic primary manifestation may also have been initiated in the bone marrow, since the pre-T cells of this compartment are destined to settle and mature in thymus and are dependent on its micromilieu. Direct initiation in the thymic cortex by irradiation from the adjacent thoracic vertebrae seems, however, to also be a plausible mechanism.

Noteworthy in this context is the absence of myeloid leukemias. Although consistent with previous experience in the same strain, this absence contrasts with the high frequencies reported in the CBA/H substrain, induced by a variety of ionizing irradiations, i.e. x-rays (39), γ-rays (40) and fission neutrons (41). A genetic difference between the two substrains and their endogenous retroviruses may account for this discrepancy. More than two decades ago Kaplan postulated that x-ray induced lymphomas in mice are mediated by a viral agent (42). The role of retroviruses in the development of radiation-induced mouse lymphomas has since been widely supported. The situation is, however, complicated by the fact that lymphomas can also be induced by irradiation in mice lacking germline ecotropic virus (43, 44).

In conclusion, the present study (parts I and II) demonstrated that when ^{90}Sr injected, young adult male CBA mice were profoundly immunosuppressed by ATx and/or ALG, they did not subsequently develop more tumours or otherwise change their tumour pattern compared with mice treated with ^{90}Sr only. This result supports the view that no simple immuno-surveillance mechanism protecting against radiogenic cancers is likely to exist. It further indicates that the immune system has no significant function in the host's defence against already established tumours, nor does it help prevent metastasis. Theoretically this means that tumour-associated antigens are either nonexistent in ^{90}Sr induced tumours or very weak and of subordinate importance in the host's defence against primary tumours. Alternatively, if such antigens exist they might either be unavailable or prevented, for other reasons, to participate in immunorejection.

Despite the lack of support for the hypothesis that the immune system has the potential to play a significant role in protecting against radiogenic cancers, any conclusion made at this time can only be tentative, owing to the limited amount of data. Thus the question remains an important one, requiring further investigation.

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