Tenfold Increased Incidence of Spontaneous Multiple Myeloma in Long-Term Immunosuppressed Aging C57BL/KaLwRij Mice

JIRI RADL,* CORNELIS VAN ARKEL,*,† CORNELIS M. HOPSTAKEN,* AND HARM HOGENESCH*

*TNO-Prevention and Health, Department of Immunology and Infectious Diseases, Leiden, The Netherlands; and †Erasmus University Rotterdam, Department of Immunology, Rotterdam, The Netherlands

Persons undergoing maintenance immunosuppressive treatment (MIST) were shown to be at increased risk for the development of early malignancies, often of cells of the immune system. Very little is known about the late effects of MIST. Some clinical studies indicated an age-related increase in the incidence of plasma-cell disorders, in particular in that of multiple myeloma (MM). In the present study the influence of MIST on the development of monoclonal B-cell proliferative disorders, monoclonal gammopathies (MG), was studied in an animal model, the C57BL/KaLwRij mouse. This strain is known for its susceptibility to develop with aging MG similar to those in humans. Two widely used treatment protocols (azathioprine/prednisolone and Cyclosporin A/prednisolone) were tested in young and adult mice. Both regiments were shown to increase 10-fold the incidence of spontaneous multiple myeloma. Unexpectedly, the same high incidence of MM and in addition the development of a life-shortening lymphoblastic lymphoma were found in a high frequency in the control group that received Cremophor EL only, i.e., the solvent of Cyclosporin A. Repeated experiments with another lot of Cremophor showed a 6-fold increased frequency of MM but no lymphoblastic lymphoma. With respect to the life-span and the incidence of hemopoietic neoplasms the least harmful drugs for MIST appeared to be azathioprine/prednisolone. The results of the experiments in this C57BL/KaLwRij mouse model give a warning for increased incidence of MM in susceptible aging individuals and address a question whether Cremophor EL is a safe solvent for Cyclosporin A. © 1996 Academic Press, Inc.

INTRODUCTION

The number of persons undergoing maintenance immunosuppressive treatment (MIST) for long periods of time (i.e., recipients of organ transplantations, some patients with autoimmune disorders) grows steadily. Short term evaluation studies suggest that they are at markedly increased risk for the development of malignancies, very often of lymphoid cells (1–4). The major-ity of these posttransplant lymphoproliferative diseases are B-cell non-Hodgkin lymphomas and are usually EBV related. Malignancies of B cells in a more mature stage are rare (5). Very little is known about the late effects of MIST. In some older studies the late effects of selected immunosuppressants on immunocompetence were studied (6–8); however, any information on the late effects of more recently introduced immunosuppressants is scarce (9). Our previous studies performed in man (10) indicated that age-related immunodeficiency, which gradually develops in individuals with genetically determined different speed, may substantially be accelerated and potentiated by MIST. We hypothesize that this will eventually lead in susceptible individuals to a more frequent development of benign and malignant proliferative disorders, mainly of B-cells.

The aim of this study was to test this hypothesis in a suitable animal model, because a follow-up investigation involving the whole life-span of an individual is necessary. The aging mice of the C57BL/KaLwRij strain offer an appropriate experimental model for such a study because these mice spontaneously develop high frequency monoclonal B-cell proliferative disorders that are similar to those in humans (11–13). Two widely used treatment protocols, azathioprine/prednisolone and Cyclosporin A (CsA)/prednisolone, were tested in young as well as in late adult mice. Both regiments were shown to increase the frequency of spontaneous multiple myeloma (MM) in mice of this strain. Control experiments using Cremophor EL, the solvent of CsA, showed the same incidence of MM and indicated strong adverse effects of this preparation.

MATERIALS AND METHODS

Mice

SPF derived female C57BL/KaLwRij mice from the colony of the TNO Institute for Experimental Gerontology in Rijswijk (presently TNO-Prevention and Health, Leiden, The Netherlands) were maintained under

clean conventional conditions. Detailed information on husbandry, health status, survival data, and age-related pathology of the strain has been published previously (14). A complete necropsy was done on mice within 2 hr of death or immediately following euthanasia of moribund animals. Histological examination of representative samples of all relevant tissues was performed according to a standard protocol. Ultrastructural examination by electron microscopy was performed on bone marrow samples in mice suspected of having multiple myeloma.

Young (4 to 5 months old) and adult (14 months old) mice of the C57BL/KaLwRij strain were first immunized with a purified human IgG1-Lambda paraprotein (KAT). Each mouse received intraperitoneally 50 μg of the protein dissolved in 0.1 ml phosphate-buffered saline (PBS) and emulsified with 0.1 ml of complete Freund's adjuvant and boosted after 4 weeks with the same amount of protein dissolved in Incomplete Freund's adjuvant/PBS (1:1). The immunization was used to obtain an additional marker for the detection of some H-Ig that were expected to develop with aging. Immunization by itself, using protein antigens and Freund's adjuvant or adjuvant alone, does not lead to the increased incidence of multiple myeloma as shown in our previous experiments (11, 15).

One month later, the mice were submitted to MIST by intraperitoneal route because administration *per os* did not guarantee an equal intake of the drugs. The mice received azathioprine (The Wellcome Foundation Ltd., London, UK) and prednisolone (prednisolone sodium succinate, N.V. Organon, Oss, The Netherlands): Group A (young mice, $n = 50$; adult mice, $n = 50$); or Cyclosporin A in solution for intravenous application (kindly donated by Sandoz AG, Basel, Switzerland) and prednisolone: Group B (young mice, $n = 50$; adult mice, $n = 47$). These drugs were given twice a week in doses corresponding to those used in human MIST as calculated according to Freireich *et al.* (16). The dosage schedule was Azathioprine, first five doses 0.9 mg each per mouse and further 0.4 mg/mouse; CsA, first six doses 3.4 mg each per mouse and further 0.72 mg/mouse; prednisolone, first dose of 0.6 mg/mouse, six following doses of 0.3 mg/mouse and further 0.12 mg/mouse, continued over the whole remaining life-spans of the mice. The average total doses received per mouse were MIST from late adult age, 27.3 mg of azathioprine and 9.0 mg of prednisolone, 47.5 mg of CsA, and 6.8 mg of prednisolone; MIST started at young age, 53.6 mg of azathioprine and 17.6 mg of prednisolone, 117.4 mg of CsA, and 17.9 mg of prednisolone.

Control groups consisted of mice receiving the solvent of the corresponding drug twice a week only, i.e., 0.25 ml PBS for azathioprine (Group D, young mice, $n = 28$; adult mice, $n = 30$) and the solvent for CsA,

Cremophor EL (Sigma Chemical Company, St. Louis, MO; lot 97F0246), 160 mg in 0.25 ml of 10% ethanol and saline (Group C, young mice, $n = 51$; adult mice, $n = 48$).

Due to unexpected results in group C, several additional experiments were performed to test the Cremophor EL influence. They, however, could only be performed with another, more recent Cremophor EL (lot 70H0361): Once a month, mice of group CR1 ($n = 15$) received, intraperitoneally, a dose of Cremophor EL (in 0.25 ml) four times higher (650 mg) than those of group C in the first experiment. The same dose was given to mice of the group CR2 ($n = 15$), but only once a week. Groups CR3 ($n = 15$) and CR4 ($n = 15$) represented the same conditions as group C in the first experiment; however, only mice of group CR4 were immunized with the human IgG1-Lambda paraprotein before starting MIST. Control mice were injected intraperitoneally with 0.25 ml PBS/10% ethanol solution, either once a month (group K1) or once a week (group K2). All mice in the second experiment were approximately 6 months of age at the start of the experiment.

Detection of Homogeneous Immunoglobulin Components

Blood samples were taken each 2 months and the sera were tested for the presence of homogeneous immunoglobulin components (H-Ig) by high resolution electrophoresis and immunoelectrophoresis (17). The appearance of H-Ig in the sera of individual mice was tentatively classified according to the following criteria (12): (1) H-Ig which progressively develop within 2 months and reach levels above 5 mg/ml. This pattern is typical for multiple myeloma spontaneously appearing in this mouse strain at the frequency of about 0.5%. (2) Distinct H-Ig with a concentration below 4 mg/ml, which persist for at least 6 months and can be detected till the death of the animals. This pattern is typical for benign monoclonal gammopathy (BMG), a benign neoplasia of B cells, which is very frequent in the C57BL/Ka mice. (3) Transient H-Ig components, usually of a low concentration, appearing for a limited period of time. These H-Ig were described as resulting from insufficient control of T-cell function in various immunodeficiencies with preserved B-cell function (including the immunodeficiency due to aging of the immune system). (4) H-Ig components which appeared shortly before the death of the mice were considered unclassifiable.

The potential specificity of H-Ig to the human IgG1-Lambda protein used for the immunization of the mice was tested by antigen-specific immunoblotting as described previously (18).

TABLE 1

Survival Analysis of C57BL/KaLwRij Mice Submitted to Two Maintenance Immunosuppressive Treatments

	(n)	χ-square	Logrank P value	Hazard ratio
Group A: Azathioprine/prednisolone[a]	young (50)	0.2023	$P > 0.2$	1.10
	adult (50)	0.4685	$P > 0.2$	1.17
Group B: Cyclosporin A/prednisolone	young (47)	15.4782[b]	$P < 0.001$	0.47
		4.2164[a]	$P < 0.01$	1.55
	adult (50)	2.0002[b]	$0.1 < P < 0.2$	0.77
		2.7998[a]	$0.05 < P < 0.1$	1.45
Group C: Cremophor EL/10% ethanol[a]	young (48)	25.06	$P < 0.001$	2.75
	adult (51)	11.8466	$P < 0.001$	2.08

[a] Compared to PBS-control group D.

[b] Compared to Cremophore EL/10% ethanol control group C.

Statistical Analysis

Survival curves were estimated by the method of Kaplan–Meier with differences assessed by the logrank test. Proportions were compared by chi-square analysis, using Yates continuity correction, or Fisher's exact test when a number was smaller than 5. P values smaller than 0.05 were considered statistically significant.

RESULTS

Survival

The life-span of mice in which MIST was started at the age of 6 months was not significantly different from the life-span of mice in which the same treatment-protocol was started at late adult age (data not shown). The treatment-protocol of group A, azathioprine and prednisolone, neither affected the life-span of young nor of adult mice as compared to the PBS-control group D (Table 1). The young immunosuppressed mice in group B had a shorter life-span compared to the PBS-treated control group. While assessing differences between the CsA/prednisolone-treated group B and the Cremophor EL-treated group C, it became apparent that young mice in the control group C had a significantly shortened life-span as compared to young mice treated with the immunosuppressants. This was not the case in adult mice, but both young and adult mice treated with Cremophor EL had a significantly shortened life-span as compared to group D ($P < .001$) (Fig. 1).

Because of these adverse effects of Cremophor EL in group C, additional experiments were performed, although with a different batch of Cremophor EL. Only when given a four times higher dose of Cremophore once a week (group CR2), the life-span of the mice was significantly shortened ($P < 0.001$; hazard ratio 2.76). None of the other groups of the second experiment showed differences in survival as compared to PBS/10%

ethanol control groups. No differences were detected in life-spans of the PBS-control group D of the first experiment and the PBS/ethanol control groups K1 and K2 in the second experiment (data not shown).
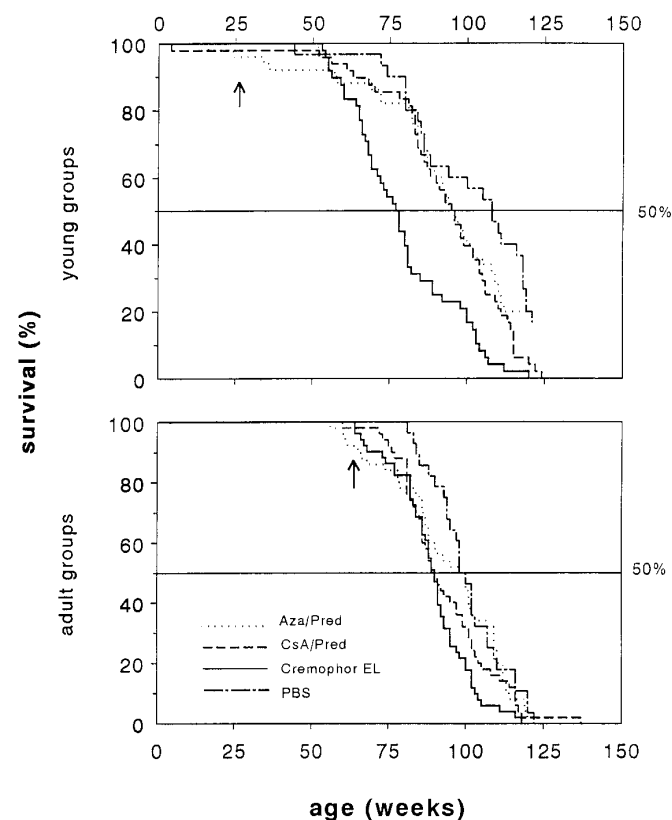

FIG. 1. Survival curves of experimental and control mice. Start of immunosuppressive treatment is indicated by arrows. The upper and lower graphs represent the groups of young mice and the groups of adult mice, respectively. A 50% survival line is shown in both graphs. Experiments with young groups A and D were terminated at 121 weeks.
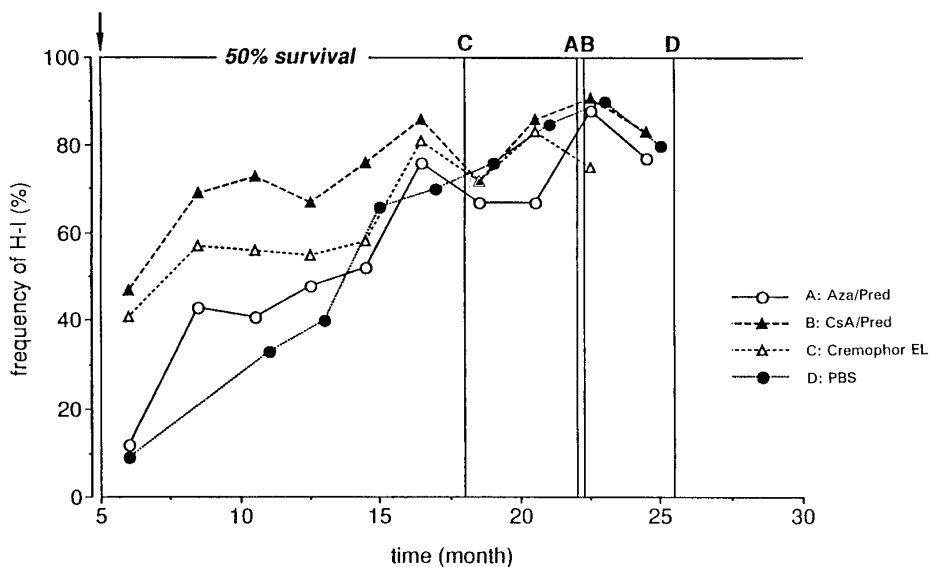
FIG. 2. Frequency curves of homogeneous immunoglobulin components (H-Ig) in mice treated from the age of 5 months with Azathioprine/prednisolone (A), CsA/prednisolone (B), Cremophor EL (C), and PBS (D) in relation to age (in months) of the mice. The 50% survival value of mice of the different groups is indicated by the vertical lines. The arrow indicates the time at which the immunosuppressive treatments were started.

Homogeneous Immunoglobulin Components

The frequency curve of H-Ig (Fig. 2) and its slope of the control mice receiving only PBS did not differ much from those in untreated normal C57BL/KaLwRij mice as seen in numerous previous experiments (12). The H-Ig frequency curve of mice receiving azathioprine/prednisolone followed more or less that of its control group, being only slightly higher in the beginning of the treatment. The frequencies of H-Ig in group C and even more in the CsA/prednisolone-treated group B were clearly higher than that of group D for several months after initiation of the treatment. At the end of the observation period, which is in fact determined by the natural ending of the lives of the animals, the frequencies of H-Ig were similar in all groups, reaching values above 70%. The characterization of the H-Ig components (Table 2) showed that the pattern of MM as detected in high resolution serum electrophoresis was seen in 5% of the mice in each of the groups A, B, and C, which was a 10 times higher incidence than was expected ($P < 0.0001$, one-proportion frequency analysis). Also transient small H-Ig components were most frequently seen in these three groups. No difference in the incidence of BMG was found. In group A the incidence of unclassified H-Ig was significantly lower than in the control group D. Over all, no differences were detected in H-Ig incidence and characteristics between groups B and C.

In the additional experiments on the effect of Cremophor EL, heavy H-Ig components with the characteristics of MM were detected in one case in group CR1 and one in group CR4. Thus the incidence of MM in the later experiments in all Cremophore groups together was 3%.

H-Ig Isotypes and Specificity

Testing the isotypes of H-Ig revealed that 11 of the 17 MM were of the IgG2b isotype. Of the remaining 6 MM, 4, 1, and 1 belonged, respectively, to the IgG2a, IgG1, and IgG3 subclasses. All of them were of the kappa light chain type. The most frequent isotype of the H-Ig in the BMG group was IgG2a. H-Ig of the transient type were of various isotypes, including IgM. There was no clear-cut H-Ig of the IgA isotype found in any of the groups.

Tests for the antibody specificity of individual H-Ig components to the antigen used for immunization revealed that one of 114 cases of BMG and one of 15 cases of MM had a positive anti-human IgG activity. The latter was a mouse (BL-59) from group A, treated with azathioprine/prednisolone. At the age of 22 months, this mouse developed an IgG2b-Kappa paraprotein that reached within the remaining 5 months of life a concentration far above 10 mg/ml (Fig. 3). The epitope on human IgG recognized by this mouse myeloma protein (as tested by a number of human IgG paraproteins of different subclasses and light chain types) was shown to belong to a common determinant on the gamma chain. Similar specificity was also found when the BMG H-Ig was tested. In none of the cases a speci-

TABLE 2

Frequency of Monoclonal Gammopathies in C57BL/KaLwRij Mice Submitted to Two
Maintenance Immunosuppressive Treatments

Experimental groups	n^a	H-Ig (%)		MM^b	BMG^b	Transient H-Ig^b	Unclassified H-Ig^b
		+	−				
A: Azathioprine/prednisolon	95	80	20	5^c	37	26^d	12^g
B: CsA/prednisolon	97	89	11	5^c	41	23^e	20
C: Cremophore EL/10% ethanol	97	89	11	5^c	36	25^f	23
D: PBS	53	87	13	0	51	6	30

[a] Number of mice suitable for evaluation.
[b] Percentage of mice with this category of monoclonal gammopathy.
[c] $P < 0.0001$ as tested in a one proportion frequency analysis, because the observed frequency of MM in the control group D was too low to apply to χ-square test we used the expected frequency of 0.5% for MM.
[d] Yates corrected P value = 0.004 when compared to group D.
[e] Yates corrected P value = 0.015 when compared to group D.
[f] Yates corrected P value = 0.007 when compared to group D.
[g] Yates corrected P value = 0.001 when compared to group D.

ficity to IgG1 subclass, to lambda light chain type, or to the KAT-protein idiotype was detected.

Histopathological Examination

Hemopoietic neoplasms were common in all experimental groups (Table 3), whereas other neoplasms were rare and randomly distributed over the experimental groups. The most common neoplasm was the follicular center cell lymphoma (FCCL), a slowly progressive malignancy of B cells (14). In mice treated from young age with CsA/prednisolone (group B), the incidence of FCCL was significantly higher as compared to group C, but was not different when compared to the PBS control group D. Lymphoblastic lymphoma (LL) occurred significantly more in the group that had received Cremophore EL, but was rare in the other treatment groups. This unexpected high incidence of LL in group C was another reason to start the additional experiment. However, there was no LL found in mice of this latter experiment.

Histiocytic sarcomas (HS) were moderately frequent in all groups. Twelve mice had more than one hemopoietic tumor: for mice had FCCL and HS, six mice had FCCL and LL, one mouse had LL and HS, and one mouse had FCCL, LL, and HS.

FCCL was most frequently found in the mesenteric lymph node, but other lymph nodes, spleen, and Peyer's patches were also commonly affected. The FCCL was characterized by nodular expansive growth and contained a mixed population of cells. The cells were small and large lymphoid cells mixed with variable numbers of plasma cells, macrophages, multinucleated cells, and granulocytes.

LL was characterized by infiltrative growth and was usually leukemic. Bone marrow involvement was common. The neoplasm was present throughout the peritoneal cavity and neoplastic cells infiltrated the abdominal viscera from the serosal surface or hematogenously. The neoplasm consisted of homogeneous population of noncohesively growing lymphoid cells with numerous mitotic figures. Neoplastic cells did not contain cytoplasmic immunoglobulin as demonstrated by immunoperoxidase histochemistry on paraffin sections (not shown).
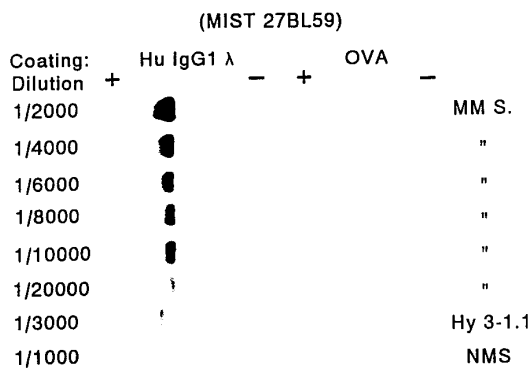

FIG. 3. Demonstration of the antibody activity to human IgG of the myeloma protein (BL59) by antigen-specific immunoblotting. The membrane on the left side was coated with the purified human IgG1-Lambda myeloma protein (KAT) used for immunization of the mice prior to start of immunosuppressive treatment; as control, coating with ovalbumin was used on the right side. Serum of the BL59 mouse (MM S.) at the age of 27 months was tested in dilutions from 1/2,000 up to 1/20,000. Normal mouse serum (dil 1/1000) and ascites of a hybridoma 3-1.1 protein (±5 mg/ml) with an activity to human IgG1 epitope (dil 1/3000) were used as negative and positive controls, respectively. Anode and katode are indicated by + and − symbols.

TABLE 3

Incidence of Hematopoietic Neoplasms in C57BL/Ka Mice Submitted to Two
Maintenance Immunosuppressive Treatments

	Experimental groups			
	A	B	C	D
Neoplasm/age of mice	(Aza/pred) No. (%)[a]	(CsA/pred) No. (%)	(Cremophore EL) No. (%)	(PBS) No. (%)
Follicular center cell lymphoma				
Young[b]	4/16 (25)	10/16 (63)[c]	2/16 (13)[d]	4/7 (50)
Adult	8/16 (50)	9/16 (56)	7/16 (44)	3/8 (43)
Total	12/32 (38)	19/32 (59)[e]	9/32 (28)	7/15 (47)
Lymphoblastic lymphoma				
Young	0/16 (0)	1/16 (6)[f]	9/16 (56)	1/7 (13)
Adult	2/16 (13)	1/16 (6)[g]	8/16 (50)[h]	0/8 (0)
Total	2/32 (6)	2/32 (6)[i]	17/32 (53)[j]	1/15 (7)
Histiocytic sarcoma				
Young	1/16 (6)	1/16 (6)	4/16 (25)	1/7 (14)
Adult	2/16 (13)	1/16 (6)	4/16 (25)	0/8 (0)
Total	3/32 (9)	2/32 (12)	8/32 (25)	1/15 (7)

[a] Number of mice with neoplasm/total number of mice in which postmortem examination was performed.
[b] Age at start of maintenance immunosuppression.
[c] Yates corrected P value = 0.011 when compared to group C.
[d] Two-sided P value = 0.045 as given by Fisher's exact test when compared to group D.
[e] Yates corrected P value = 0.023 when compared to group C.
[f] Yates corrected P value = 0.008 when compared to group C.
[g] Two-sided P value = 0.015 as given by Fisher's exact test when compared to group C.
[h] Two-sided P value = 0.022 as given by Fisher's exact test when compared to group D.
[j] Two-sided P value = 0.006 as given by Fisher's exact test when compared to group D.

HS was primarily present in the liver, lung, lymph nodes, and uterus. The tumor consisted of ovoid or spindle-shaped histiocytic cells. Multinucleated cells and erythrophagocytosis were common.

MM was characterized on light microscopy by the presence of multiple foci of pleomorphic plasmacytoid cells without distortion of the normal architecture of the bone marrow or osteolysis. These were most commonly found in the pelvic bones and in the distal femur and proximal tibia. Ultrastructural examination demonstrated typical plasmacytoid cells with abundant rough endoplasmic reticulum and a well-developed Golgi apparatus.

DISCUSSION

Short term studies evaluating the effects of MIST on the development of neoplasias showed an increased risk of the treated persons for malignancies, very often of lymphoid cells (1–5). Only little is known about the late effects of long-lasting MIST. Literature review (5) and preliminary data from our studies on kidney recipients (10) indicated increased frequencies of MM and BMG that were related more to the higher age than to the duration of MIST. This led to the hypothesis that MIST accelerates and potentiates the development of an age-related immunodeficiency and eventually leads in susceptible individuals to benign and malignant neoplasias mainly of the B cells. This hypothesis seems to be corroborated by the present experiments for at least the increased incidence of MM. A 10-fold increase in the incidence of MM was observed in both treatment regimens. The incidence of BMG, a benign B-cell neoplasia, was not increased. However, BMG is a typical phenomenon of old age and therefore, the shortened survival of the mice in some of the experimental groups may have influenced the results.

The frequency distribution of isotypes within H-Ig of the different categories was similar with that seen in other experiments (11, 12). While the most frequent isotype of H-Ig in BMG was IgG2a, in MM, typically, the IgG2b isotype was clearly dominant. An interesting finding was the anti-IgG specificity of one MM and one BMG to the protein used for immunization prior to the MIST initiation. Both paraproteins recognized a common determinant on the human IgG1L protein. This indicated that both BMG and MM developed from B-cell clones responding to a specific antigenic stimulation, even very long before the disorder developed. As also our other previous experiments indicated (11, 15), it may be the memory B cell that becomes target for oncogenic events.

As far as the other malignancies are concerned, there was no significant difference in the occurrence of HS among the four groups. Young mice in group C had the lowest incidence of FCCL, even less than control group D. Since FCCL is a disease of old age, the shortened survival of these mice can explain this finding. Although malignant LL was detected in a few mice of the MIST-treated groups and once in the PBS-control group D, 53% of mice ($P < 0.006$, Table 3) in the Cremophore control group C had histologically confirmed LL.

The effect of Cremophor EL unexpectedly complicated the whole experiment. Due to its highly carcinogenic effect (MM, LL) in the first experiment, it was further tested in additional experiments. There, the incidence of MM was found to be 3%, but that of LL was not increased. The second lot of Cremophore EL diminished the survival probability significantly only in high doses (experiment 2, data not shown). The only plausible explanation of the events leading to the development of malignant LL in a high frequency and causing the shortened survival of the mice, would be the presence of an unknown contaminant in the first lot of Cremophor EL with carcinogenic properties. Our search for some additional information on the previous lot of Cremophore EL remained unsuccessful. It may well be that the effect of Cremophore EL in the first experiment was an example of (co-) clastogenic effects of Cremophore, which is the enhancement of genotoxicity by Cremophore in the presence of a carcinogenic substance (19). The mechanism by which Cremophore, a derivative of Castor-oil and ethylenoxide, enhances carcinogenicity is not known. It is used as a solvent for hydrophobic drugs, such as CsA and paclitaxel. It possibly facilitates carcinogenic substances to cross the cell membranes. The incomplete reversal of multiple drug resistancy by Cremophore may also be a mechanism by which this solvent induces cytotoxicity or even malignant transformation (20). Our observation stresses the importance of well-defined control groups. While Cremophore is being used as a solvent for intravenous application only for short period in the beginning of MIST, it itself or its derivatives used for peroral treatment may not be innocent solvents, especially when carcinogenic contaminants could be present in some preparations.

In this study using a mouse model, azathioprine/prednisolone were shown to be the least harmful drugs for MIST when considering life-span and incidence of hematopoietic neoplasms. Only the incidence of MM was increased, but this was also the case in the CsA/prednisolone-treated group. In this latter group, it is not clear whether Cremophore contributed to the increased incidence of MM and this should further be studied. In humans, the question should be addressed whether the genetically determined increased susceptibility for the development of MM could be predicted and consequently any long-term immunosuppression protocols could be avoided or adapted to minimize the risks.

REFERENCES

1. Penn, I., Tumors of the immunocompromised patient. *Annu. Rev. Med.* 39, 63–73, 1988.

2. Penn, I., The changing pattern of posttransplant malignancies. *Transplant. Proc.* 23, 1101–1103, 1991.

3. Cockburn, I., Assessment of the risks of malignancy and lymphomas developing in patients using Sandimmune. *Transplant. Proc.* 19, 1804–1807, 1987.

4. Hanto, D. W., and Simmons, R. L., Lymphoproliferative diseases in immunosuppressed patients. *In* "Progress in Transplantation" (P. J. Morris and N. L. Tilney, Eds.), Vol. 1, pp. 186–208, Churchill-Livingstone, Edinburgh, 1984.

5. Joseph, G., Barker, R. L., Yuan, B., Martin, A., Medeiros, J., and Peiper, S. C., Posttransplant plasma cell dyscrasias. *Cancer* 74, 1959–1964, 1994.

6. Perkins, E. H., Peterson, W. J., Gottlieb, C. F., Halsall, M. K., Cacheiro, L. H., and Makinodan, T., The late effects of selected immunosuppressants on immunocompetence, disease incidence, and mean life-span. I. Humoral immune activity. *Mech. Ageing Dev.* 4, 231–240, 1975.

7. Peterson, W. J., Perkins, E. H., Goodman, S. A., Hori, Y., Halsall, M. K., and Makinodan, T., The late effects of selected immunosuppressants on immunocompetence, disease incidence, and mean life-span. II. Cell-mediated immune activity. *Mech. Ageing Dev.* 4, 241–250, 1975.

8. Peter, C. P., Perkins, E. H., Peterson, W. J., Walburg, H. E., and Makinodan, T., The late effects of selected immunosuppressants on immunocompetence, disease incidence, and mean life-span. III. Disease incidence and life expectancy. *Mech. Ageing Dev.* 4, 251–260, 1975.

9. Peest, D., Schaper, B., Nashan, B., Wonigeit, K., Raude, E., Pichlmayr, R., Haverich, A., and Deicher, H., High incidence of monoclonal immunoglobulins in patients after liver or heart transplantation. *Transplantation* 46, 389–395, 1988.

10. Radl, J., Valentijn, R. M., Haaijman, J. J., and Paul, L. C., Monoclonal gammapathies in patients undergoing immunosuppressive treatment after renal transplantation. *Clin. Immunol. Immunopathol.* 37, 98–102, 1985.

11. Radl, J., Monoclonal B-cell proliferative disorders and aging. *In* "Physiological Senescence and Its Postponement: Theoretical Approaches and Rational Interventions" (W. Pierpaoli and N. Fabris, Eds.), *Ann. N.Y. Acad. Sci.,* Vol. 621, pp. 418–427, 1991.

12. Radl, J., Aging and proliferative homeostasis: Monoclonal gammopathies in mice and men. *Lab. Anim. Sci.* 42, 138–141, 1992.

13. Radl, J., The C57BL/KaLwRij mouse model in the studies of monoclonal gammapathies. *In* "Multiple Myeloma and Related Disorders" (F. Dammacco and B. Barlogie, Eds.), Series Challenges of Modern Medicine, Vol. 4, pp. 11–17, Ares-Serono Symposia Publications, Rome, 1994.

14. Van Zwieten, M. J., Zurcher, C., Solleveld, H. A., and Hollander, C. F., Pathology. *In* "Immunological Techniques Applied to Aging Research" (W. H. Adler and A. A. Nordin, Eds.), pp. 1–36, CRC, Boca Raton, 1981.

15. Nooij, F. J. M., Van der Sluijs-Gelling, A. J., and Radl, J., Development of aging-associated monoclonal gammapathies with antibody activity to the antigen used for immunization of young mice. *Clin. Immunol. Immunopathol.* 63, 110–114, 1992.

16. Freireich, E. J., Gehan, E. A., Rall, D. P., Schmidt, L. H., and Skipper, H. E., Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother. Rep.* 50, 219–223, 1966.

17. Radl, J., Effects of aging on immunoglobulins. *In* "Immunological Techniques Applied to Aging Research" (W. H. Adler and A. A. Nordin, Eds.), pp. 121–139, CRC, Boca Raton, 1981.

18. Nooij, F. J. M., Van der Sluijs-Gelling, A. J., Jol-van der Zijde, C. M., Van Tol, M. J. D., Haas, H., and Radl, J., Immunoblotting techniques for, the detection of low level homogeneous immunoglobulin components in serum. *J. Immunol. Methods* 134, 273–281, 1990.

19. Au, W. W., Anwar, W., Paolini, M., Ramanujam, S., and Cantelli-Forti, G., Mechanism of clastogenic and co-clastogenic activity of cremophore with benzene in mice. *Carcinogenesis* 12, 53–57, 1991.

20. Fallskog, M-L., Frii, L., and Bergh, J., Is Cremophor EL, solvent for paclitaxel, cytotoxic? *Lancet* 324, 873, 1993.