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The Cellular Basis of Immunosuppression Caused by the Radiation Leukaemia Virus

A. PELED AND N. HARAN-GHERA

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel

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Summary. Infection of adult C57Bl/6 mice with the radiation leukaemia virus resulted in suppression of the ability of the animals to respond to an immunizing inoculum of sheep erythrocytes. Results of the transfer experiments indicated that the immunosuppressive effect was expressed at the immunocompetent cell level, and that the virus affected the thymus-derived population of immunocytes. The immunosuppressive effect of the virus on thymus cells, independent of any contribution by cells of bone marrow origin, was verified with thymus-independent immunogens, polyvinylpyrrolidone (PVP) or pneumococcal polysaccharide SIII (PPS). Mice inoculated with the radiation leukaemia virus produced nearly normal amounts of plaque-forming cells producing antibodies against PVP and PPS, thereby confirming that the immunosuppressive effect of the radiation leukaemia virus was on thymus-derived cells.

INTRODUCTION

In our previous work (Peled and Haran-Ghera, 1971), we showed that the immune response of C57Bl/6 mice following intra-thymic injection of the radiation leukaemia virus was impaired; a marked depression in humoral and cellular response to sheep erythrocytes (SRBC) was demonstrated. These results coincided with those of several investigators, who studied the immunosuppressive effect of a number of leukaemogenic viruses (Peterson, Hendrickson and Good, 1963; Cremer, Taylor and Hagens, 1966; Ceglowski and Friedman, 1968). The mechanism by which the murine leukaemia viruses suppress the immune response is not yet clear. Several investigators have proposed that virus and antigen compete for a stem cell that has immunoproliferative potential (Siegel and Morton, 1966; Ceglowski and Friedman, 1967). Friedman and Ceglowski (1968) have shown, using the cell transfer technique, that spleens of mice infected with Friend leukaemia virus (FLV) were deficient in cells that could respond to antigen when transferred to an irradiated syngeneic host. Both the time interval between injection and assay and the virus dose determined the degree of the suppressive effect. More recently Bennett and Steeves (1970) have studied the interaction of thymus and bone marrow cell mixtures from normal and FLV-injected donors for their ability to restore the immune response to SRBC in heavily irradiated syngeneic hosts. They observed a marked suppression in the bone marrow precursors of antibody-forming cells, while thymus cells collected from virus-injected mice showed no functional impairment.

The aim of the present study was to determine whether the reduction in the immune

response of C57Bl/6 mice following inoculation of the radiation leukaemia virus could be attributed to thymus and/or bone marrow-derived lymphocyte populations.

MATERIALS AND METHODS

Animals

Male and female inbred mice of the C57Bl/6 strain from the Institute Animal Breeding Centre were used when 5–8 weeks old. The animals were kept in an air conditioned room at 21–25° and fed purine laboratory chow and tap water *al libitum.*

Radiation leukaemia virus

The radiation leukaemia virus preparations were obtained from the thymic tissue of C57Bl/6 mice with lymphoid leukaemia induced by serial passage lines of the virus, originally isolated from the bone marrow of irradiated isogeneic animals (Haran-Ghera, 1966). The leukaemia thymus was homogenized in 5 volumes of chilled phosphate-buffered saline (PBS). The homogenate was centrifuged three times for 15 minutes at 10,000 ***g***, the pellet being discarded each time. The entire process was carried out at 4°. The virus preparation used in the present study termed 'passage 136', induces lymphatic leukaemia in 80–100 per cent of young adult mice (following its inoculation (0·02 ml) directly into the thymus) at an average latent period of 110 days.

X-irradiation

Mice were exposed to a single dose of 700 rad total body irradiation from a Maximar 250 III General Electric instrument, 230 kV 15 ma, with 1 mm AL and 0·5 mm Cu filters; dose rate: 45 rad/minute.

Antigens and immunization

Sheep red blood cells (SRBC) were stored in Alsever's solution at 4°. The mice were injected intraperitoneally with 0·5 ml of 10 per cent washed SRBC in saline. Polyvinyl-pyrrolidon (PVP-K_{90}), mol. wt 360,000 (Fluka AG, Switzerland) was dissolved in phosphate-buffered saline (PBS) at pH 7; immunization was carried out by an intravenous injection of 0·25 μg of PVP. Pneumococcal polysaccharide SIII (PPS) (Wellcome) was dissolved in saline and injected intraperitoneally, the dose used being 0·6 μg/mouse.

The response to SRBC was tested at day 4 after immunization, whereas the responses to PVP and PPS were tested at day 5 after antigen injection.

Plaque assay

The number of spleen cells producing antibodies to SRBC was assessed by the agar plaque technique of Jerne and Nordin (1963). The number of plaques (PFC) per spleen was calculated.

The response to PVP was tested by the haemolytic plaque assay using SRBC coated with PVP K15 (Fluka AG, Switzerland, mol. wt 10,000). Fresh, washed SRBC in PBS were incubated at room temperature for 10 minutes in 0·01 per cent tannic acid in PBS at a ratio of 1:1. After several cell washings the packed tanned cells were incubated for another 10 minutes at room temperature in 0·1 mg of PVP K15/ml PBS. Thereafter, the coated SRBC were washed three times in PBS, adjusted to 5 per cent in PBS and used for the plaque assay.

Packed washed SRBC were suspended in 1·0 ml of saline containing 1000 μg of pneumococcal polysaccharide SIII, and 1·0 ml of 0·1 per cent chromium chloride (anhydrous, B.D.H.) in saline was added to the mixture. After 5 minutes of incubation at room temperature, the erythrocytes were washed a few times with saline and were adjusted to a final concentration of 5–10 per cent (Baker, Stashak and Prescott, 1969).

Cell suspensions and transfers

C57Bl/6 mice were injected intraperitoneally (0·5 ml) with the radiation leukaemia virus 'passage 136' or with PBS (the solution used for virus preparation). One month later, suspensions of thymus and bone marrow cells were prepared in Tyrode solution. 5×10^7 thymocytes were mixed with 2×10^7 bone marrow cells (total volume 0·5 ml) and 0·5 ml of 10 per cent SRBC was added to the mixed cell suspension; the total of 1 ml volume was injected intravenously into syngeneic recipients that had been exposed to 700 rad whole body irradiation a few hours earlier. The following cell combinations were tested: (1) normal thymus (NT) and bone marrow (NB) cells from PBS-treated mice; (2) thymus cells from normal donors with bone marrow cells from virus-injected hosts (VB); (3) thymocytes from virus-injected donors (VT) with NB; (4) thymus and bone marrow cells both from virus-inoculated mice; (5) NB only; (6) without donor cell transfer. The spleens from the different treated groups were removed 8 days after the cell transfer, and the number of plaque-forming cells per spleen was evaluated.

RESULTS

Effect of the virus on thymus and bone marrow cells in response to SRBC

In our previous work (Peled and Haran-Ghera, 1971), we showed that injection of the virus directly into the thymus of adult C57Bl mice resulted in a marked decrease in their immune response to SRBC. In order to eliminate the effects of possible thymus damage due to injecting the virus into the thymus, the organ used in the following transfer experiment, we tested whether intraperitoneal injection of the virus would cause similar immune impairment. A significant reduction in the number of PFC was obtained when 0·5 ml of virus was injected intraperitoneally (the test being done 30 days following virus inoculation), the results obtained being 100 ± 21 PFC per spleen in the virus inoculated mice compared to 485 ± 52 PFC/spleen in the matching controls injected with PBS (the medium used for virus injection).

In order to establish whether the virus-induced suppression to SRBC could be attributed to an effect on immunocompetent cells, thymus and bone marrow cells from virus-injected and/or PBS-injected donors mixed with SRBC were transferred into irradiated, syngeneic, non-virus-treated recipients. The response of the repopulated spleens to SRBC was evaluated. The results are summarized in Table 1. The number of PFC/spleen in irradiated mice reconstituted with normal thymus (NT) and normal bone marrow cells (NB) taken from PBS injected donors was 1753 ± 329. Normal thymus cells (NT) mixed with bone marrow cells from virus-inoculated donors (VB) showed a similar response (1136 ± 282—the difference being not significant). A significant reduced response was obtained when thymus cells from virus-inoculated donors (VT), were mixed with either normal bone marrow cells harvested from PBS-injected donors, obtaining 245 ± 53 PFC/spleen or with bone marrow cells from virus-injected donors (VB), 441 ± 165 PFC/

TABLE 1

THE EFFECT OF VIRUS ON THYMUS AND BONE-MARROW CELLS IN RESPONSE TO SRBS

Cell combination injected:	Direct PFC/spleen ± S.E.
5×10^7 NT + 2×10^7 NB	1752 ± 329
5×10^7 NT + 2×10^7 VB	1136 ± 282
5×10^7 VT + 2×10^7 VB	441 ± 165
5×10^7 VT + 2×10^7 NB	245 ± 53
NB	82 ± 33
No cells infected	16 ± 8·5

C57Bl/6 mice, 3 months old, were exposed to 700 rad whole body irradiation; within 1–3 hours after irradiation they received an intravenous injection of one of the different combinations of thymus and bone marrow cells taken from normal (injected with PBS) or virus-injected donors (30 days after 0·5 ml intraperitoneal injection of virus or PBS). SRBC (0·5 ml) was injected simultaneously with the cell suspension. Spleens were tested 8 days after cell transfer. Fifteen mice were used in each group.

NT, thymus cells from normal hosts (injected with PBS). NB, bone marrow cells from normal hosts (injected with PBS). VT, thymus cells from-virus injected mice. VB, bone marrow cells from virus injected mice. PFC, plaque-forming cells. S.E., standard error.

spleen, namely a significant difference ($P<0·005$) in comparison to the controls (1753 ± 329 PFC/spleen).

The results obtained imply that the thymus-derived cells were affected by the radiation leukaemia virus.

Immune response of virus-injected adult mice to thymus-dependent and thymus-independent immunogens

In the previous experiment we demonstrated the impaired immune response of adult C57Bl mice injected with the radiation leukaemia virus, using SRBC—a thymus-dependent immunogen, and indicated that only thymus-derived cells were affected by the virus. It seemed, therefore, of interest to test the immune response of virus-inoculated mice to thymus-independent immunogens in comparison to SRBC. The thymus-independent immunogens used were: polyvinylpyrrolidone (PVP) (Andersson and Blomgren, 1971)

TABLE 2

THE SUPPRESSIVE EFFECT OF THE VIRUS ON THE RESPONSE TO THYMUS-DEPENDENT AND THYMUS-INDEPENDENT ANTIGENS

Host treatment*	Direct PFC/spleen ± S.E.					
	SRBC		PVP		PPS	
PBS	89,300 ± 8202	$P<0·005$	7920 ± 744	N.S.	2306 ± 478	N.S.
'Passage 136' Virus	21,000 ± 3785		8400 ± 754		1922 ± 612	

* Female C57Bl/6 mice, 8 weeks old, were injected with either 0·02 ml of PBS (phosphate-buffered saline), or 'passage 136' virus, directly into one thymus lobe. 30 days later, 0·5 ml of 10 per cent SRBC, or 0·26 μg of PVP K_{90} or 0·6 μg of pneumococcal polysaccharide SIII was injected intraperitoneally. Ten mice were used in each group.

SRBC, sheep red blood cells. PFC, plaque-forming cells. S.E., standard error. PVP, polyvinylpyrrolidone. PPS, pneumococcal polysaccharide SIII. *P*, level of probability (Student's *t*-test). N.S., statistically not significant.

and pneumococcal polysaccharide SIII (PPS) (Davies, Carter, Leuchars, Wallis and Dietrich, 1970). The radiation leukaemia virus or PBS (0·02 ml) was injected directly into one thymus lobe of C57Bl mice. One month later, these inoculated mice were immunized with either PVP, PPS or with SRBC. On the fourth day after immunization with SRBC, or on the fifth day after immunization with PVP or PPS, the spleens of the treated mice were removed and the number of plaque-forming cells against coated SRBC or SRBC was evaluated. The results are summarized in Table 2.

The response to SRBC in the PBS-injected control mice, was 89,300±8202 PFC/spleen whereas in the virus-inoculated mice a decrease in the number of PFC/spleen was observed—21,000±3,785. In contrast, when the virus-injected mice were immunized with the thymus-independent immunogens PVP or PPS, the mice produced nearly normal amounts of PFC. The number of PFC/spleen was almost the same in the PBS- or virus-injected mice: 7920 PFC/spleen versus 8400 in the PVP test group and 2306 PFC/spleen compared to 1922 in the mice immunized with PPS. These results indicate that the immunosuppressive effect of the radiation leukaemia virus involves thymus-derived cells.

DISCUSSION

Following the observation of reduced immune response to SRBC after radiation leukaemia virus injection, we wondered whether this immunosuppressive effect could be demonstrated at the immunocompetent cell level, being attributed to a thymus and/or a bone marrow-derived population of immunocytes. The cell transfer technique (Mitchell and Miller, 1968), that demonstrated the presence or absence of specific antigen-sensitive and/or antibody-forming cells, was chosen to study whether the leukaemogenic virus interferes with the function of stem cells or antibody synthesizing cells. The results obtained imply that the thymus-derived cells *per se* were affected by the virus, since their immunocompetent function was impaired. It should be stressed that histological examination of thymuses taken 30 days after virus inoculation did not reveal the presence of leukaemic cells in them. This observation eliminates the possibility of producing a shift in the cell population of the thymus, so that immunocompetent cells are still immunocompetent, but they are diluted out by the increase of non-responsive leukaemic cells. An additional proof of the selective activity of the radiation leukaemia virus on thymus cell function was presented by using two thymus-independent immunogens, polyvinylpyrrolidone (PVP) and pneumococcal polysaccharide SIII (PPS). The response of virus-injected mice immunized with SRBC was markedly decreased in comparison to normal controls, whereas injected mice immunized with PVP or PPS responded similarly to the normal matching controls—namely, the immunosuppressive effect of the virus was not reflected in the bone marrow-derived population of the immunocytes.

The present findings, indicating that thymus-derived cells were markedly suppressed by the radiation leukaemia virus, contrast with the observations of Bennett and Steeves (1970), who found that the immunosuppressive effect of Friend virus on antibody response to sheep erythrocytes could be attributed to the marrow-derived but not to the thymus-derived population of immunocompetent cells. These dissimilar results might be understood if we consider Thomson's observations (1969), that bone marrow cells serve as target cells for Friend virus replication and our recent findings (Haran-Ghera

and Peled, 1973), that the lymphatic leukemia induced by the virus is of thymus-derived lymphocyte origin. Recent studies concerned with the cellular analysis of virus-induced immunosuppression in the SJL/J strain of mice (Shearer, Mozes, Haran-Ghera and Bentwich, 1973), indicated that both thymus-derived helper cells and bone marrow-derived precursors of antibody cells were affected by the virus injection. It should be mentioned that the immunogen used in these studies was a multichain synthetic polypeptide, whereas SRBC was used in all the other studies mentioned above. The susceptibility of SJL/J mice to develop different types of leukaemias (Haran-Ghera, Kotler and Meshorer, 1967), might explain this double effect of the virus (or perhaps viruses?) on thymus and bone marrow-derived cells.

The immunosuppressive effects associated with tumourigenic viruses have been of particular interest, since some investigators have proposed a correlation between the changes in the immune response caused by the virus and the onset of tumour development. In our previous studies (Peled *et al.*, 1971), we found no correlation between the degree of immunosuppression caused by the radiation leukaemia virus and its leukaemogenic activity. Recently Haran-Ghera, Ben-Yaakov, Peled and Bentwich (1973) have shown that in the SJL/J strain of mice, there was a decrease in the immune reactivity with age increase (irrespective of whether the host was normal or tumour bearing), but this defective immunological status did not seem to play a role in spontaneous tumour development.

The virus–immunocompetent cell interaction, indicated in the present study, should be taken into account in considering the aging effects on the immune response, since the leukaemogenic virus is present during post-natal life in many strains of mice and its titre increases with aging (Haran-Ghera and Peled, 1967).

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