A-4.4 Epidemiological and experimental studies for the effects of UV-B irradiation on the immune response and infectious diseases

Contact person Kiichi Yamamoto, Senior researcher
Department of Virology I,
The National Institute of Infectious Diseases,
The Ministry of Health and Welfare,
1-23-1 Toyama, Shinjuku-ku, Tokyo 162, Japan
Tel:+81-3-5285-1111, Fax:+81-3-5285-1188

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[A] A trial introducing epidemiological method in the fields of immune response and infectious diseases.

[Abstract]
In this study, to clarify the effect of UV-B exposure from sunlight on immune responses epidemiologically, we analyzed the data of tuberculin-reaction tests (TRT) on infants and students. The TRT-positive rate notably declined from north to south, dependent on latitude. This trend was reversely relative to increasing UV-B irradiation from the north to the south of Japan.

[Key words] Tuberculin-reaction test (TRT), latitude, UV-B exposure level, negative correlation

[Introduction]
Epidemiological evidence for the effect of UV irradiation on immune responses or infectious diseases is rare. Since immune responses are generally not visual, when immunological effects of UV irradiation are investigated, biological samples such as blood should be obtained and examined for immune functions. Recently, it has become ethically difficult to obtain samples for the purpose of epidemiological studies.

On the other hand, infectious diseases which is diagnosable only by clinical symptoms without experimental inspections are limited. Usually, it takes several days for the inspection. Due to this complicated procedure, surveillance of common infectious diseases is not usually performed at hospitals or clinics except during outbreaks. Therefore, it is limited for useful information to introduce epidemiological methodology into the fields of immune responses or infectious diseases. To fulfill this objective, and to clarify the effect of UV-B irradiation on immune responses epidemiologically, we analyzed the data of tuberculin-reaction tests (TRT) on infants and students.

[Materials and Methods]
TRT data used Table 25 (Medical inspect receiving number for tuberculosis) in the "Report on Activities of Health Centers" by the Statistics and Information Department of the Ministry of Health and Welfare [1]. In this study, data from 1995 was selected. The TRT-positive ratio was calculated as the percentage of TRT-positive infants and students to total number surveyed in this medical inspection. For the estimation of TRT-positive ratio by latitude, Japanese islands were grouped into 8 blocks of several prefectures from north to south. The regional UV-B

levels were referred to in a published paper [2]. The significance between north and south positive ratios was calculated with a specific method termed the "Trend Line in Appearance Ratio". By this method, it was estimated to be significant when the "U" value was over 3.32 (p=0.1%).

[Results and Discussion]

The mean TRT-positive ratio is summarized in Table 1, and visualized on the Japanese prefectural map. The positive ratio (%) decreased from north (Hokkaido) to south (Kyushu) corresponding to latitude (Fig 1, Table 1). Since the U value was 36.8 the decline in TRT positive ratio is significant (Table 1).

Conversely, as shown in Table 1, the mean regional UV-B level of each block increased depending on north to south movement. Evaluation of the association between the TRT-positive ratio and the amount of UV-B estimated values showed a negative correlation in all areas except Okinawa. While Okinawa is located the furthest south, and the UV-B exposure level is the highest (2,321x10J/m^2), the TRT-positive ratio was also the highest (59.5%). In this study, we could not clarify why Okinawa jumped to the highest TRT level.

TRT is a delayed -type hyper sensitivity which is a type of cellular immunity. It is known that UV irradiation affects cellular immunity, but not humoral immunity. The negative-correlation between TRT positive ratio and regional UV-B level might be regarded as a good example that experimental findings can reflect field's data in outdoor.

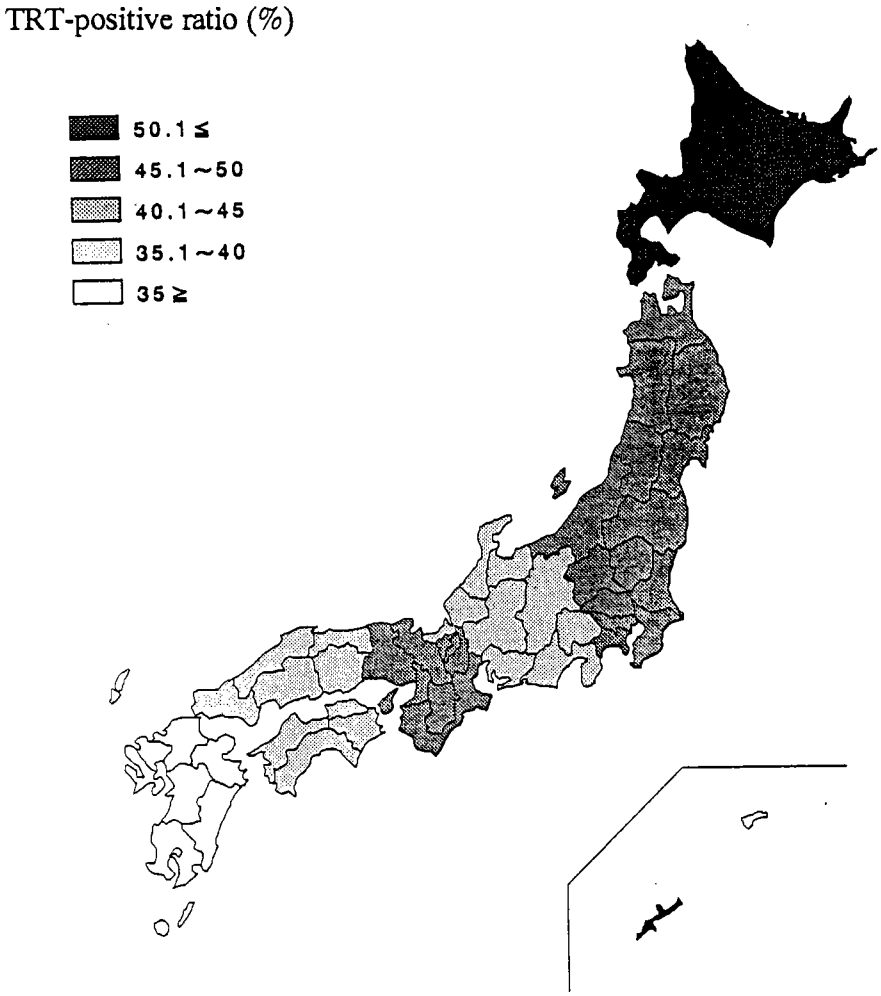
[References]

1. The Protection of Tuberculosis. In "Report on Activities of Health Center" eds. Statistics and Information Department. pp. 100. (1995)
2. Takahashi K., Kawana K., Okubo T. et al., Estimation of Regional UV-B Levels as Exposure Indices for Ecological and Environmental Epidemiologic Studies, J. UOEH 18: 51-60 (1966).

Table 1. Correlation between the estimated UV-B level and TRT-positive ratio

Block of prefecture	UV-B (10 J/m^2)	TRT-positive ratio (%)
Hokkaido	1045.0	53.7
Tohoku	1355.1	41.8
Kanto	1492.8	42.0
Koshin・Chubu	1634.6	35.8
Kinki	1625.0	40.4
Chugoku・Shikoku	1754.9	35.7
Kyushu	1799.0	34.1
Okinawa	2320.9	59.5
		U=36.8 (p=0.1 %)

Fig. 1 Changes of TRT-positive ratio of infants and students by latitude in Japan



[B] Harmful effect of UV-B exposure on infectious diseases.

[Abstract]

UV-B irradiation extensively affected malarial infection of mice. In susceptible BALB/c strains, pre-irradiation of UV-B enhanced susceptibility, and in resistant C57BL/10 strains, the mice became susceptible after UV irradiation. The immunological cause of the change from resistant to susceptible depended on the decline of IFN- γ by UV irradiation.

[Key words] UV-B, Murine malaria, IFN- γ .

[Introduction]

A body of evidence has been accumulating over the past 20 years indicating that the exposure of humans and experimental animals to UV-B irradiation can modify certain immune

responses, suggesting greater susceptibility to infectious diseases. The United Nations of Environmental Programme (UNEP) reports that malaria is an important diseases in terms of increasing the risk of infection due to ozone depletion and the resulting UV-B increase on earth[1]. However, the referring source was based on a personal communication and to date, no epidemiological or experimental reports have been published demonstrating that UV-B irradiation enhances malarial infection.

In this study, using a mouse model, we investigated whether UV-B is a risk factor for malarial infection.

[Materials and Methods]

Female BALB/c and C57BL/10 mice, 7-10 weeks old, were used. After dorsal hair was removed with clippers and depilatory cream, mice were irradiated once with 200 mJ/cm^2 of UV-B ray. Murine malaria (Plasmodium chabaudii) was injected intraperitoneally (ip.) at 10^4 or 10^5 parasitized red blood cells (PRBC). A serum sample from each group of mice was collected by heart puncture. IFN-γ was assayed by ELISA kit for murine IFN-γ.

[Result]

After BALB/c mice, a strain susceptible to murine malaria, were challenged with a sublethal dose of 10^4 PRBC, 4 of 5 mice survived. However, when the mice were UV-B pre-irradiated, all mice died within 10 days (Fig. 1A). C57BL/10 mice, a strain resistant to murine malaria, survived even after challenge with 10^5 PRBC. When pre-irradiated with UV-B, however, all mice died within 11 days (Fig. 1B).

The plasma IFN-γ titers of unirradiated control mice began to increase 4 days post-infection, reaching a maximum (mean titer, 412 pg/ml) at 5 days and then rapidly decreasing on the 6th day (Fig. 2). In contrast, the plasma IFN-γ titers of irradiated mice were low and the maximum was about one-fifth (81 pg/ml) of the control.

[Discussion]

The effect of UV-B irradiation on the susceptibility of two mouse strains to murine malaria was investigated. UV-B irradiation enhanced the susceptibility of BALB/c to mouse malaria and all mice died even at a sublethal doses of infection. Surprisingly, UV-B irradiation converted the resistant characteristic of C57BL/10 to susceptible. Because the mean survival times were within the same time frame as in BALB/c, it was supposed that the death of C57BL/10 by UV-B irradiation might have progressed through the same pathological mechanism as that of the susceptible BALB/c mice.

The most crucial difference between BALB/c and C57BL/10 mice is considered to be the production of IFN-γ, the levels being low in BALB/c and high in C57BL/10 [2]. The plasma levels of IFN-γ in C57BL/10 increased after infection with murine malaria and reached a maximum on day 5. In contrast, IFN-γ levels were reduced by UV-B irradiation before infection. Although the values for 3 mice per group were widely dispersed, the difference between the groups was statistically significant. From this result, it was supposed that the susceptiblity of C57BL/10 by UV-B irradiation was induced by the decline of IFN-γ.

IFN-γ is a cytokine secreted from T helper 1 (Th 1) cells [3]. Therefore, it is supposed that UV-B down-regulates Th 1-dependent immune responses. It is yet to be determined what causes this inhibition. This mouse model may be useful for risk assessment of increased UV-B exposure due to ozone depletion on human susceptibility to infectious diseases.

[References]
1. Van der Leun, J.C., Takizawa Y. and Longstreth J.D. (1989) Chapter 2. Human Health. In "Environmental Effects Panel Report" eds. UNEP. pp. 11-24, Nairobi, Kenya.
2. Stevenson, M.M., Tam, M.F. and Nowotarski, M.E. (1990) Role of interferon- γ and tumor necrosis factor in host resistance to *Plasmodium chabaudi* AS. Immunol. Lett. 25, 115-122.
3. Mosman, T., Cherwinski, H., Bond, M.W. et al. (1986) Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activity and secreted proteins. J. Immunol. 136, 2348-2357.

Fig. 1 Effect of UV-B irradiation on susceptibility of mice to malarial infection

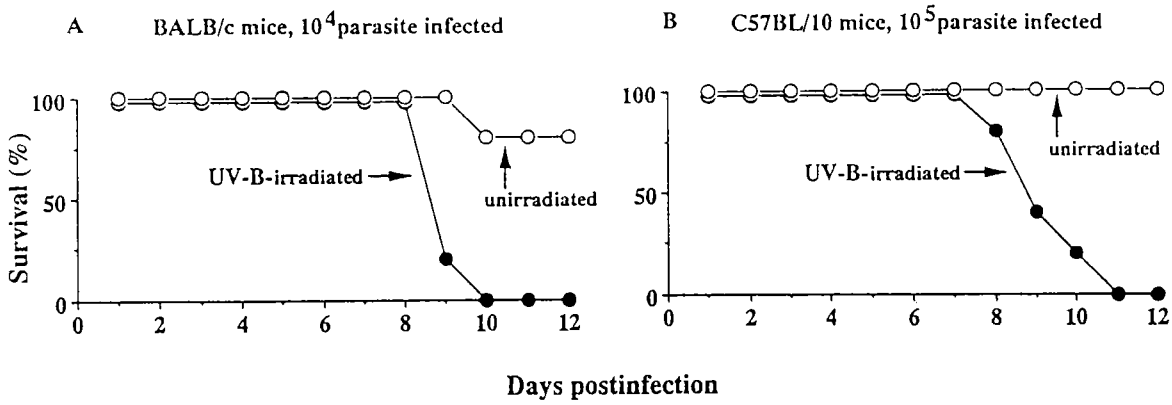
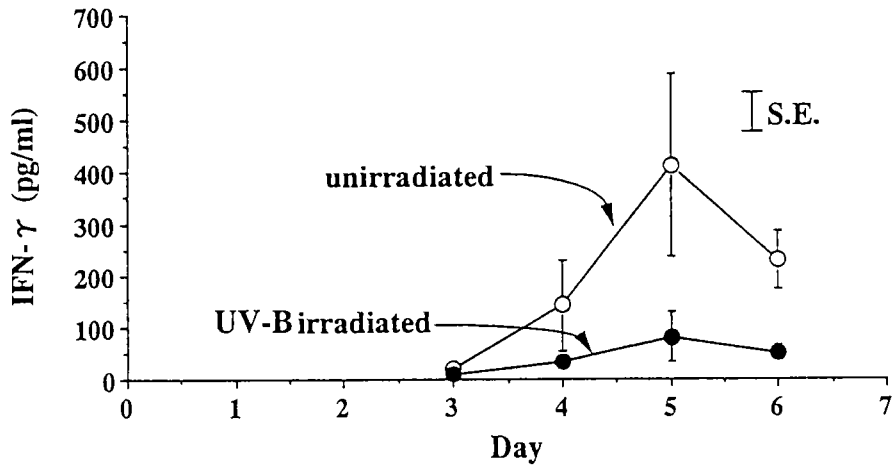


Fig. 2 Effect of UV-B irradiation on IFN- γ production of malaria-infected mice



[C] Proposal of a new branch "Photohematophysiology"

[Abstract]

Several factors in mouse blood were greatly changed after UV-B irradiation, in particular a dominant increase in haptogrobin and hemopexin which are constituents of plasma sialoglycoprotein. The plasma sialic acid-increase following UV-B irradiation depended on the increase in both sialoglycoproteins, but not other plasma proteins such as α_1-acid glycoprotein. Plasma lipoprotein changed the plasma levels and the electrophoretic properties in which low density lipoprotein greatly increased in density from trace amounts in control following UV-B irradiation, and high density lipoprotein was retarded in the mobility with a broadening of the band. The blood of mice exposed to UV-B showed fast and complete coagulation, in which free red blood cells were not seen.

[Background]

In the course of experimental study on the effects of UV-B exposure on immune responses or infectious diseases, we obtained a few samples in which several factors in mouse blood were greatly changed after UV-B irradiation described in the Abstract. The changes might not correlate directly with immune responses or infectious diseases. However, it is possible that the activating mediator in this phenomena is similar to the mediator acting in immuno-modulating reactions following UV-B irradiation. Therefore, through these studies, we expect to progress in our understanding of the immuno-modulating reactions following UV-B irradiation. We suppose that our observation might be a small part of the whole. In view of this background, we propose to establish a new research branch "Photohematophysiology". In this section, the most dramatic change, the effect of UV-B irradiation on plasma lipoprotein, was picked-up and reported from several changes summarized in the Abstract.

[1] Effect of UV-B irradiation on lipoprotein in mouse plasma

[Key Words] UV-B, High density lipoprotein, Low density lipoprotein

[Introduction]

In the acute phase reaction following UV-B irradiation, hepatocytes in the liver are activated, releasing increased levels of acute phase reactants into plasma[1)2)]. Since lipoproteins are synthesized in the liver, inducement of the acute phase might also modulate the level of plasma lipoproteins. In the present study, we investigated the effects of UV-B irradiation on lipoprotein metabolism in mice.

[Materials and Methods]

Mice (female ddy strain; age 12 weeks) were UV-B irradiated at 240 mJ/cm^2 and bled on the third day. The sera were directly applied to agarose gels and after electrophoresis, the lipoprotein bands were visualized by cholesterol-staining.

[Result]

UV irradiation had no effect on the serum concentration of high density lipoprotein (HDL) but the electrophoretic mobility of the lipoprotein band was slower in UV irradiated mice than in the unirradiated control mice (Fig.1). Furthermore, the retarded band was broadened widely.

In control mice, the low density lipoprotein (LDL) was hardly detectable, but after UV irradiation the LDL concentration was markedly increased by about 8 fold, and the LDL band showed a slight reduction in electrophoretic mobility. These changes were associated with UV strength and were reversible, since the mobility of lipoprotein bands returned to normal on the 8th day after UV irradiation (data not shown).

[Discussion]

Baumann et al. found that apolipoprotein A1 was a negative reactant [3]. On the other hand, we found that UV irradiation markedly increased the plasma level of LDL and retarded the HDL with a broadening of the band in electrophoresis. The increase in LDL shows a unique side effect of UV rays as a risk factor for human and animal health. Whether our findings indicate a direct change in structure or metabolism of lipoproteins due to an acute phase reaction, it is yet to be determined.

[References]

1. Yamamoto K., Takano K. and Yamamoto M. Increase of plasma sialic acid upon UV-B irradiation in mouse. Biol. Pharm. Bull. 18: 917-919 (1995).
2. Yamamoto K., Furuya T., Kameoka Y. and Kawanaka M. Changes in serum levels of sialoglycoproteins in mice exposed to UV-B radiation. Biol. Pharm. Bull. 21: 1000-1002 (1998)
3. Baumann H., Jahreis G.P. and Gaines K.C. Synthesis and regulation of acute phase plasma proteins in primary cultures of mouse hepatocytes. J. Cell Biol. 97: 866-976 (1983).

Fig. 1 Electrophoretic pattern of plasma lipoproteins from UV-B irradiated mice