Reduced T Cell Response in Carcinogen-sensitive Donryu Rats Compared with Carcinogen-resistant DRH Rats

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Carcinogen-resistant DRH rats were developed from carcinogen-sensitive Donryu rats, which showed a high incidence of hepatic tumors when they were exposed to 3′-methyl-4-dimethyl-aminoazobenzene (3′-MeDAB) or other aminoazo hepatocarcinogens. In order to study the mechanism of the difference of carcinogenesis, we studied the immunological competence of Donryu rats compared with that of DRH rats. Anti-keyhole limpet hemocyanin (KLH) antibody and KLH-specific delayed hypersensitivity (DTH) responses after immunization with KLH were reduced in Donryu rats compared with DRH rats. Proliferative responses of spleen cells to KLH and nonspecific mitogens such as conconavalin A (Con A) and phytohemagglutinin (PHA) were significantly lower in Donryu rats than in DRH rats. Upon the cross-linking of T cell receptor (TCR) complex using anti-CD3 monoclonal antibody (Mab), spleen cells from Donryu rats proliferated poorly. Two other strains of rats, SD and Wistar, exhibited high responsiveness, comparable to that of DRH rats, indicating that the responsiveness of Donryu rats was impaired. The production of interleukin-2 (IL-2) upon stimulation with Con A and the responsiveness of Con A blasts to exogenous IL-2 were also attenuated in Donryu rats. In contrast to T cell responsiveness, natural killer (NK) cell activity of spleen was increased in Donryu rats. Flow cytometric analysis revealed that the expression of CD4 and CD8 on T cells was decreased in Donryu rats, though the expression of other T cell markers such as CD2, CD3 and CD8 was not different. These results indicate that Donryu rats, which have been used in many years for cancer research in Japan, have impaired immunological surveillance mechanisms. This is likely to be one of the factors accounting for the high sensitivity to chemical carcinogens and the high susceptibility to transplanted tumor cells of Donryu rats.

Key words:    Donryu rat — DRH rat — Immunological competence — T cell — NK cell

Carcinogenesis is a complex event which is initiated by exposure to carcinogens and may be prevented by the immune surveillance mechanisms of the hosts.1 Doryu rats have been used for a long time for cancer research in Japan, because these rats are very sensitive to hepatocarcinogens2, 3 and are susceptible to transplanted tumor cells.4, 5 Yoshimoto et al. have developed a new strain of carcinogen-resistant DRH rats from carcinogen sensitive Donryu rats over more than 20 generations.2, 6 DRH rats show marked resistance to the potent hepatocarcinogen 3′-MeDAB, 4-dimethyl-aminoazobenzene, and the mammary carcinogen 7,12-dimethylbenz(α)anthracene, compared with Donryu rats.2, 3 Several studies have been carried out to examine the metabolic activities in the liver which induce conversion of carcinogens to their ultimate carcinogenic form, such as the induction of mRNA of cytochrome P450 enzyme, which functions as a terminal oxidase of 3′-MeDAB, the activity of hydroxysteroid sulfotransferase, which may catalyze the final step of activation of 3′-MeDAB, and so on. The carcinogen-resistant DRH rats show reduced ability to metabolize carcinogens.2, 3, 6, 7 Although the immune surveillance mechanism of the host plays an important role during carcinogenesis, the immunological functions of these rats remain to be determined. Therefore, in this study, we compared the immunological competence of carcinogen-sensitive Donryu and resistant DRH rats.

MATERIALS AND METHODS

Animals Male Donryu and DRH rats were purchased from Seac Yoshitomi Ltd. (Fukuoka) and used at 7 to 12 weeks of age for the experiments.

Monoclonal antibodies (Mabs) and polyclonal antibodies The following Mabs were used in this study: anti-CD2 (MRC Ox-34, murine IgG2a, Serotec Ltd., Oxford,
England), anti-CD3 (1F4, murine IgM, Serotec Ltd.), anti-CD4 (W3/25, murine IgG1, Serotec Ltd. and RTH-7, murine IgG2a, Seikagaku Kogyo Co., Tokyo), anti-CD5 (R1-3B3, murine IgG2a, Seikagaku Kogyo Co.) and anti-CD8 (MRC OX-8, murine IgG1, Serotec Ltd. and R1-10B5, murine IgG2a, Seikagaku Kogyo Co.). The following polyclonal antibodies were also used: alkaline-phosphatase-conjugated goat anti-rat IgG Fc (Chemicon International Inc., Temecula, CA), FITC-conjugated goat anti-rat IgG (Organon Teknika Co., Turnhout, Belgium), FITC-conjugated goat anti-mouse IgG (H+L) absorbed with IgG fraction of rat serum (Cedarlane Laboratories, Ontario, Canada) and FITC-conjugated goat anti-mouse IgM absorbed with IgM fraction of rat serum (Organon Teknika Co.).

**Immunization of rats** Donryu and DRH rats were immunized intraperitoneally with 100 μg of KLH (Sigma Chemical Co., St Louis, MO) with complete Freund’s adjuvant (Difco Laboratories, Detroit, MI).

**Titration of anti-KLH antibody** Four weeks after the immunization, sera were harvested and anti-KLH antibody was determined by ELISA. Microtiter plates (Immulon 4, Dynatech Laboratories Inc., Chatilly, WI) were coated with 10 μg/ml KLH at 37°C for 2 h and at 4°C overnight. They were blocked with 1% bovine serum albumin (Sigma Chemical Co.), and serially diluted rat sera were added. The plates were incubated at 37°C for 1 h and bound IgG was detected with alkaline-phosphatase-conjugated anti-rat IgG antibody using p-nitrophenyl phosphate (Nacalai Tesque Inc., Kyoto) as a substrate. The absorbance at 405 nm was measured using an ImmunoMini NJ-2300 (Inter Med Co., Tokyo). The results were expressed as mean±SE of antibody titer (2nd serum dilution).

**Detection of DTH** Four weeks after the immunization, rats were challenged with 50 μg of KLH intracutaneously and the size of induration was measured 24 h after the challenge. The results were expressed as mean diameter±SE (mm).

**Assay of proliferative responses** A spleen cell suspension was prepared from normal rats and treated with 0.83% NH4Cl in 0.017 M Tris buffer (pH 7.2) to lyse red blood cells. Spleen cells (2×10⁵) were cultured in 0.2 ml of RPMI 1640 medium (Nissui Seiyaku Co., Tokyo) containing 10% FCS (Biowhittaker, Walkersville, MD) and 5×10⁻⁵ M 2-mercaptoethanol in a flat-bottomed microtiter culture plate (Falcon #3072, Becton Dickinson and Co., Lincoln Park, NJ) at 37°C in 5% CO₂ and 95% air for 5 days with 10 μg/ml KLH, Con A (Sigma Chemical Co.) or PHA (Difco Laboratories). The cells were pulsed with 0.5 μCi of [³H]thymidine (specific activity, 5 mCi/mm, Amesham, Buckinghamshire, England) for the last 15 h and then harvested on glass-fiber filters with the aid of a cell harvester (Abe Kagaku Co., Chiba). The incorporation of [³H]thymidine into spleen cells was measured by a liquid scintillation counter (Aloka Co., Tokyo). Cross-linking of CD3 was performed in the following way. Thirty microfilters of anti-CD3 Mab solution (5 or 10 μg/ml) was added to a round-bottomed microtiter culture plate (Falcon #3077). The plate was incubated at 37°C for 90 min and then washed three times with ice-cold phosphate-buffered saline, pH 7.2, before used. Spleen cells (2×10⁶) were cultured in the plate for 3 days as described above.

**Assays of the production of IL-2 and the responsiveness of Con A blasts against exogenous IL-2** Spleen cells (1.5×10⁶/3 ml) were cultured in RPMI 1640 medium containing 10% FCS in the presence or absence of 10 μg/ml Con A at 37°C in 5% CO₂ and 95% air for 24 h. Then, the culture supernatant was harvested by centrifugation. The recovered cells were further cultured for 3 more days and used as Con A blasts. IL-2 concentration in the culture supernatant was measured using an IL-2 assay ELISA kit (Immuo Biological Laboratories Co., Fujikoka). The responsiveness of Con A blasts to exogenous IL-2 was assayed in the following manner. Con A blasts (5×10⁴) were cultured with 100 units/ml of recombinant human IL-2 (donated by Dr. J. Hamuro, Ajinomoto Co., Tokyo) in RPMI 1640 medium containing 10% FCS in a flat-bottomed microtiter culture plate (Falcon #3072) at 37°C in 5% CO₂ and 95% air for 3 days. The cells were pulsed with [³H]thymidine for the last 15 h and the incorporation of [³H]thymidine by Con A blasts was measured as described above.

**T cell purification and assay of accessory cell function** Purified T cells were prepared by passing spleen cells through a nylon wool column. Briefly, 10⁵ spleen cells were applied to a column containing 1 g of nylon wool and incubated at 37°C for 1 h. Nylon-nonadherent cells were eluted and used as T cells. Spleen cells were incubated with 100 μg/ml mitomycin C (Kyowa Hakko Kogyo Co., Tokyo) at 37°C for 1 h, washed and used as accessory cells. Then 2×10⁵ purified T cells and 10⁵ accessory cells were cultured with or without Con A or PHA at 37°C for 4 days and [³H]thymidine incorporation by the T cells was studied as described above.

**Assay of NK cell activity** NK cell activity was assayed using spleen cells without the treatment with 0.83% NH₄Cl and B cell lymphoma, Yac-1, as target cells. Yac-1 cells (4×10⁴) were radiolabeled by incubating them in 2 ml of RPMI 1640 medium containing 10% FCS and 100 μCi of [⁵¹Cr]sodium chromate (specific activity, 200 mCi/mg Cr, Amersharm) at 37°C for 60 min. The Cr-labeled Yac-1 (1×10⁴) were washed and cultured with graded numbers of spleen cells (1×10⁶, 0.5×10⁶, 0.25×10⁶) in 0.2 ml of RPMI 1640 medium containing 10% FCS in a round-bottomed microtiter culture plate (Falcon #3077) at 37°C for 5 h. After the incubation, the culture supernatant was harvested and the released radioactivity was counted with a γ counter (Aloka Co.). The percentage of cell lysis
was calculated from the following formula: % specific lysis = [(experimental release – control release)/(maximum release – control release)] × 100, where the maximum release was obtained by incubating 10Cr-labeled Yac-1 cells in the presence of 1% Triton X-100 (Sigma Chemical Co.) and control release was obtained by incubating target cells without spleen cells.

**Production of TNF by peritoneal macrophages** Resident peritoneal macrophages (5×10⁵/ml) were cultured with or without 10 μg/ml LPS (Sigma Chemical Co.) at 37°C in 5% CO₂ and 95% air for 6 h. The TNF activity in the culture supernatant was assayed by using L929 cells.¹³ L929 cells (10⁴/0.1 ml) in RPMI 1640 medium containing 5% FCS were cultured in a flat-bottomed microtiter culture plate (Falcon #3072) at 37°C for 20 h. Then, the culture medium was replaced with serially diluted TNF samples and the cells were further cultured at 37°C for 20 h in the presence of 1 μg/ml of actinomycin D (Sigma Chemical Co.). After the culture, the culture medium was discarded and the cells were stained with 0.2% crystal violet in 20% methanol (Sigma Chemical Co.) for 15 min. The plates were washed with tap water, the cells were solubilized with 1% sodium dodecyl sulfate (Sigma Chemical Co.), and the absorbance at 540 nm was measured with an ImmunoMini NJ-2300. The percentage cytotoxicity was calculated as (1 - Aₛ₅₀ of sample/Aₛ₅₀ of control) × 100. The results were expressed as units of TNF produced by 5×10⁵ macrophages compared with recombinant human TNF (donated by Dr. M. Yamada, Dainippon Pharmaceutical Co., Osaka) as the standard.

**Flow cytometric analysis of T cell markers** Spleen cells (2×10⁵) were incubated with 10% normal goat serum, to block Fc receptor, on ice for 30 min. The cells were washed and incubated with 10 mM phosphate buffer-0.15 M NaCl containing an optimal concentration of antibody. The first step reagent was anti-CD2, anti-CD3, anti-CD4, anti-CD5 or anti-CD8 Mab. The second step reagent was FITC-conjugated anti-mouse IgG or anti-mouse IgM antibody. To detect B cells, spleen cells were directly stained with FITC-conjugated anti-rat IgG antibody. Viable cells (10,000/sample) were analyzed by an Excel flow cytometer (Coulter Electronics Inc., Hialeah, FL).

**Statistics** All experiments were repeated at least 3 times and representative results are shown in the tables and figures. Statistical analysis was performed by using Student’s t test. A confidence level of <0.05 was considered significant.¹³

**RESULTS**

**Antibody and DTH responses after immunization were reduced in Donryu rats** To study the immunological functions of carcinogen-sensitive Donryu rats and carcinogen-resistant DRH rats, we immunized both rats with a protein antigen, KLH, and the anti-KLH antibody response in their sera and KLH-specific DTH response were compared. As shown in Fig. 1, both antibody and DTH responses were significantly lower in Donryu rats than in DRH rats. These results suggest that some immunological abnormalities exist in Donryu rats.

**Reduced T cell functions in Donryu rats compared with DRH rats** To analyze more precisely the immunological functions, we studied the proliferative responses of spleen cells from these rats. As shown in Table I, the proliferative response of spleen cells to stimulation with KLH was reduced in Donryu rats compared with DRH rats. Furthermore, the responses of spleen cells to nonspecific mitogens such as Con A and PHA were also significantly lower in Donryu rats than in DRH rats. In the following experiments we mainly used nonspecific mitogens. We also examined the responsiveness of spleen cells from two other strains of rats, SD and Wistar. The proliferative responses of spleen cells from these two strains of rats were high, comparable to those from DRH rats (data not shown). These results suggest that the responsiveness of spleen cells, especially T cell responses, is reduced in Donryu rats. To confirm this point, the proliferative response of spleen cells stimulated by cross-linking of TCR complex using plate-bound anti-CD3 Mab was assayed. As shown in Fig. 2, the proliferative responses of Donryu rats were significantly lower than those of DRH rats.
Next, we compared IL-2 production of spleen cells stimulated with Con A. The results showed that the production of IL-2 of spleen cells from Donryu rats was also lower than that of DRH rats (Fig. 3A). The responsiveness of Con A blasts to exogenous IL-2 was also examined. Con A blasts from Donryu rats proliferated poorly in response to IL-2 compared with those of DRH rats (Fig. 3B). These results indicate that the activation and the differentiation of T cells of Donryu rats were impaired.

Accessory cell function of spleen cells from Donryu rats was not impaired. T cell activation with antigens or mitogens requires accessory cells such as dendritic cells and macrophages. Therefore, the reduced T cell functions of Donryu rats may be due to the reduced functions of accessory cells. In order to study this possibility, purified T cells from each rat were mixed with mitomycin C-treated spleen cells from each rat as accessory cells. As shown in Table II, T cell function from Donryu rats was not restored by the presence of accessory cells from DRH rats. Furthermore, accessory cells from Donryu rats stimu-

**Table I. Proliferative Response of Spleen Cells from Donryu and DRH Rats**

| Exp. No. | Strain of rats | KLH | Con A | PHA |
|----------|----------------|-----|-------|-----|
| I        | Donryu         | 1.637±371 | 1.868±294 | 3.173±502 | 7.146±1.918 |
|          | DRH            | 1.220±401 | 8.373±587 | 29.732±6.165 | 28.370±3.253 |
| II       | Donryu         | 1.835±159 | 2.045±238 | 18.060±4,388 | 7.492±760 |
|          | DRH            | 1.637±591 | 3.390±582 | 72.542±11,277 | 53,155±7,069 |

a) Spleen cells (2×10⁶) from Donryu or DRH rats which had been immunized with KLH 4 weeks previously were cultured with 10 µg/ml KLH, Con A or PHA at 37°C for 5 days. The cells were labeled with 0.5 µCi of [³H]thymidine for the last 15 h, harvested, and the radioactivity incorporated into the cells was counted. The results were expressed as mean±SD of [³H]thymidine incorporation in triplicate cultures. * Statistically significant difference between Donryu and DRH rats.
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lated DRH T cells as well as DRH accessory cells. These results indicate that the reduced T cell function of Donryu rats is derived from T cells themselves, but not from accessory cells.

**Study of surface markers of T cells** Next, we studied surface markers of T cells using flow cytometry. Fig. 4 shows a representative fluorescence profile of spleen cells stained with anti-CD4 Mab, RHT-7 or anti-CD8 Mab, R1-10B5, from Donryu (solid line) and DRH rats (dashed line). As can be seen, in Donryu rats, the fluorescence intensities of CD4 and CD8 were lower than those of DRH rats. Table III shows the percentages and MFIs of various T cell markers such as CD3, CD4, CD8, CD2 and CD5. Again, the MFI of CD4 was significantly lower in Donryu rats than in DRH rats. The MFI of CD8 of Donryu rats was also lower than that of DRH rats, though this was not statistically significant. On the other hand, MFIs of other T cell surface markers such as CD3, CD2 and CD5 were not different between Donryu and DRH rats. When spleen cells were stained with Mabs from other clones (anti-CD4 Mab, W3/25, and anti-CD8 Mab, MRC OX-8), spleen cells of Donryu rats also showed weaker staining than those of DRH rats (data not shown). However, thy-mocytes of Donryu rats were stained with a similar intensity to those of DRH rats (data not shown). Therefore, the difference of MFI of CD4 or CD8 in spleen cells may be ascribed to a difference of the amount of CD4 or CD8 expressed on the cell surface, but not to a difference of affinity of CD4 or CD8 for the Mab. Although the percentage of CD4-positive cells was higher in spleen cells of Donryu rats, there is no significant difference in the CD4/CD8 ratio between Donryu and DRH rats. The percentage

Table II. Accessory Function of Spleen Cells for T Cell Activation Is Not Different between Donryu and DRH Rats

| Responder T cells | Accessory cells | [3H]Thymidine incorporation (cpm) of T cells stimulated with |
|------------------|----------------|----------------------------------------------------------|
|                  |                | — | Con A | PHA |
| Donryu           | Donryu         | 124±10 | 3,070±850 | 2,115±725 |
| DRH              | Donryu         | 185±17 | 1,284±167 | 1,483±450 |
| DRH              | DRH            | 946±26* | 10,682±487* | 9,976±331* |

|                  |                | 954±159* | 5,578±419* | 11,081±908* |

*a) Nylon column-purified T cells (2×10⁵) from Donryu or DRH rats were cultured with mitomycin C-treated spleen cells (10⁵) as accessory cells from Donryu or DRH rats in the presence or absence of 10 μg/ml Con A or PHA for 4 days and [3H]thymidine incorporation by T cells was counted. Mitomycin C-treated spleen cells alone from Donryu and DRH rats showed 266±109 and 255±125, respectively. * Statistically significant difference between Donryu and DRH T cells.

Fig. 4. Expression of CD4 and CD8 on spleen cells. Spleen cells (2×10⁵) from Donryu (solid line) or DRH (dotted line) rats were treated with anti-CD4 or anti-CD8 Mab, followed by fluoresceinated goat anti-mouse IgG antibody. A representative fluorescence profile of each antibody is shown.
and MFI of surface Ig-positive cells tended to be increased in Donryu rats, but there is no significant difference between Donryu and DRH rats. **NK cell activity was increased in Donryu rats** We next examined the NK cell activity of spleen cells using Yac-1 as target cells. Spleen cells of Donryu rats exhibited higher activity than that of DRH rats (Fig. 5). Statistical significance, however, was only observed at the effector-to-target cell ratio of 100:1. **Cytokine production of peritoneal macrophages** We finally examined macrophage functions by studying TNF production by peritoneal macrophages stimulated with LPS. As shown in Fig. 6, TNF production by macrophages from Donryu rats was variable among rats, and there is no significant difference between Donryu and DRH rats. Other cytokines such as IL-1 also gave similar results (data not shown). These results suggest that there is no difference of macrophage functions between Donryu and DRH rats.

**DISCUSSION**

T cells from carcinogen-sensitive Donryu rats exhibited lower proliferative responses to antigen, mitogens and anti-CD3 Mab, compared with those from carcinogen-resistant DRH rats. This suggests that T cell functions of Donryu rats are reduced, rather than those of DRH rats being increased, because T cells from other strains of rats such as SD and Wistar proliferated comparably to those from DRH rats. In Donryu rats, T cell differentiation was also impaired, because the responsiveness of Con A blasts to exogenous IL-2 was also attenuated. Reduced T cell function of Donryu rats was not due to reduced accessory cell functions of Donryu rats, because accessory cells of Donryu rats stimulated T cells from DRH rats as well as DRH accessory cells did. Concomitantly with reduced T cell functions in vitro, antibody and DTH responses in vivo were also reduced in Donryu rats. The reduced T cell responses seem to be due to the reduced expression of CD4 and CD8 on T cells in Donryu rats. It is known that

![Graph showing NK cell activity of spleen cells from Donryu and DRH rats.](image)

**Table III. Surface Markers of Spleen Cells from Donryu and DRH Rats**

|        | Donryu | DRH |
|--------|--------|-----|
| CD3    | 41.2±3.7 | 46.2±1.3 |
| CD4    | 37.5±1.9 | 31.0±0.8* |
| CD8    | 19.1±1.6 | 17.2±1.2 |
| CD2    | 46.4±3.9 | 47.0±0.8 |
| CD5    | 47.4±3.4 | 52.6±3.3 |
| IgG    | 42.9±3.8 | 37.2±4.6 |

*Statistically significant difference between Donryu and DRH rats.

**Fig. 5.** NK cell activity of spleen cells from Donryu (●) and DRH (○) rats. The assay of NK cell activity was performed by incubating spleen cells with 51Cr-labeled Yac-1 cells at the indicated effector-to-target cell ratios for 5 h. The result is expressed as mean±SE of % specific cytotoxicity against Yac-1 cells of 3 rats. * Statistically significant difference between Donryu and DRH rats.

**Fig. 6.** TNF production of peritoneal macrophages stimulated with LPS. 5×10^5 macrophages from Donryu or DRH rats were cultured with or without 10 µg/ml LPS for 6 h. The TNF activity of culture supernatant was measured using L929 cells. The results were expressed as units of TNF produced by 5×10^5/ml macrophages compared with recombinant human TNF as the standard. Bar shows mean±SE of 5 rats. No significant difference was observed between Donryu and DRH rats.
CD4 and CD8 molecules bind to MHC class II or class I molecules, to function as a co-receptor for antigen recognition and activation of T cells. Interaction of anti-CD4 Mab or gp160 molecules from HIV with CD4 molecules inhibits the proliferation of T cells induced by CD3 cross-linking, mitogens or IL-2. It is reported that a physical association between CD4 or CD8 and TCR complex is barely detectable in resting murine T cells. On the other hand, after TCR triggering, this association is induced and two CD4 molecules associate with each TCR. In the present study, in contrast to CD4 and CD8, there is no difference in the intensity of CD3 expression between Donryu and DRH rats. Although it is not known how CD4 molecules of Donryu rats associate with TCR upon TCR triggering, it is conceivable that CD4 down-expression itself may result in poor proliferative responses.

Reduced expression of CD4 was also achieved by infection of CD4+ T cells with HIV, simian immunodeficiency virus, myxoma virus, human herpesvirus 7, or human T-cell leukemia virus-1. Many viral products have been shown to interact directly with non-receptor protein tyrosine kinase, causing disturbed signal transduction. Nef protein produced by HIV or simian immunodeficiency virus also interacts with p56lck, which is associated with the cytoplasmic domain of CD4, resulting in CD4 down-regulation. In Donryu rats, tyrosine phosphorylation or intracellular Ca2+ mobilization is also attenuated. However, the reduced T cell responsiveness in Donryu rats should not be ascribed to virus infection, because F1 hybrid rats exhibited the same phenotype as that of DRH rats (data not shown).

It is reported that both CD4+ and CD8+ T cells are important in eradicating tumor cells. CD4+ T cells are more important, especially in the early phase of immune response to tumor cells. Several studies have been carried out to examine the mechanism of the different carcinogen sensitivity of Donryu and DRH rats, focusing on the different metabolic activity for hepatocarcinogens such as 3′-MeDAB. However, the reduced responsiveness of T cells in Donryu rats concomitantly with the decreased expression of CD4 and CD8 may also be an important influence on the sensitivity to several carcinogens. This is supported by the fact that Donryu rats are not only highly sensitive to chemical carcinogens, but also highly susceptible to transplanted tumor cells. It has been reported that more than 90% of Donryu rats die after inoculation of only 10–100 hepatoma cells, such as Yoshida sarcoma. We could not compare the susceptibility of Donryu and DRH rats to transplanted tumor cells. Donryu rats died within 15 days after the intraperitoneal injection of 5×10⁶ AH66 hepatoma cells, but DRH rats did not (data not shown). These two types of rats may be not syngeneic, because a mixed culture of spleen cells from Donryu and DRH rats induced proliferative responses. Furthermore, mutual skin grafts were rejected within 12 days (data not shown).

In contrast to T cell response, NK cell activity was higher in Donryu rats than DRH rats. This is similar to the case of T cell-deficient nude mice, in which NK cell activity is much higher than that of normal mice. There is no difference of macrophage functions, such as accessory function for T cell activation and cytokine production, between Donryu and DRH rats.

In conclusion, we present evidence that there are immunological abnormalities, especially in T cell lineage, in carcinogen-sensitive Donryu rats. These abnormalities may result in the disturbance of immune surveillance mechanisms against the development of tumors. This may be one of the reasons for the high sensitivity of Donryu rats to various carcinogens.

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