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Effect of whole-body x-irradiation on antigen-induced airway response in sensitized guinea pigs

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ABSTRACT: The objectives of this study were to test the hypothesis that x-irradiation inhibits the late asthmatic response (LAR) without influencing the early asthmatic response (EAR) and to examine the mechanism of the inhibitory effect.

Twenty sensitized guinea pigs were irradiated at a dose of 8 Gy. The next day, one-half of the animals were injected intravenously with spleen cells (2×10^8) collected from unirradiated sensitized guinea pigs, whilst the other half were injected with vehicle only. Ten additional unirradiated sensitized guinea pigs also received vehicle only. Antigen inhalation challenge took place two days later. Pulmonary resistance was measured for 6 h after antigen exposure, and bronchoalveolar lavage and lung fixation were then undertaken. The area under the percentage pulmonary resistance curve 2–6 h after allergen inhalation was used for analysis of the LAR, while the maximal percentage change in pulmonary resistance was used for analysis of the EAR.

Irradiation abolished the LAR (364.4±49.4 *versus* 62.8±10.4) without inhibiting the EAR (229.3±27.2 *versus* 278.7±40.2) and significantly inhibited the accumulation of eosinophils and lymphocytes in the airways. Transfer of spleen cells restored the LAR (334.4±66.8) and the recruitment of cells to the levels seen in unirradiated sensitized guinea pigs. In addition, transfer of only CD4+ T-lymphocytes separated from the spleen cells restored the LAR (439.4±62.1) and the cell infiltration into the airways.

These inhibitory effects of x-irradiation were due to decreases in numbers of CD4+ T-lymphocytes.

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Antigen inhalation challenge produces acute airway obstruction (the early asthmatic response, or EAR), which is often followed after 4–12 h by a second airway obstruction (the late asthmatic response; LAR) [1]. While the EAR is generated by immunoglobulin (Ig)E-mediated activation of mast cells to release chemical mediators [2], the LAR depends on airway inflammation, which is generally characterized by the infiltration of eosinophils and lymphocytes into the airways [3, 4]. Inflammatory cell progenitors originating from bone marrow are also thought to be involved in the airway inflammation of the LAR [5]. Although bone marrow as a source of inflammatory cells is known to be sensitive to x-irradiation [6], as are leukocytes (especially lymphocytes) [7], the function and numbers of mast cells are not influenced even by lethal doses of x-irradiation [8, 9].Thus, x-irradiation could conceivably inhibit the LAR without influencing the EAR at all.

A guinea pig model of bronchial asthma has been developed in which both the EAR and the LAR follow allergen challenge [10]. As previously reported, the LAR in this model is associated with histological changes, such as infiltration of eosinophils and mononuclear cells into the bronchial lumen wall, as well as the contraction of airway smooth muscle, the development of oedema of the airway wall, and the formation of mucus plugs in the bronchial lumen.

In the present study, using the model, it was examined whether whole-body x-irradiation blocks the LAR without affecting the EAR. In addition, the effects of adoptive transfer of sensitized guinea-pig spleen cells in general, and of CD4+ T-lymphocytes in particular, on the restoration of the LAR were evaluated. To the authors′ knowledge, this is the first report that x-irradiation completely blocks the LAR without affecting the EAR at all.

Methods

Animals

Specific-pathogen-free male Hartley guinea pigs (Charles River, Kanagawa, Japan) weighing 160–180 g were housed in the animal research facility of Tohoku University School of Medicine. Animal care and handling were in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals" [11]. The experimental protocols described were approved by the Animal Care and Use Committee of Tohoku University.

Measurement of pulmonary resistance

The method of measuring pulmonary resistance (R_L) has been described previously [12, 13]. In brief, a pneumotachograph coupled to a differential pressure transducer was connected to the endotracheal tube cannulated into a trachea to measure airflow; volume data were obtained by electrical integration of the flow signal with an integrator. Oesophageal pressure was measured with a water-filled polyethylene tube and a pressure transducer. Airway opening pressure was measured with a pressure transducer connected between the endotracheal tube and the pneumotachograph. The R_L during spontaneous breathing for each animal was calculated by the method of MEAD and WHITTENBERGER [14].

Irradiation

Whole-body irradiation was generated with a therapy machine (model SHT250M-3; Shimazu, Tokyo, Japan), which was operated at 200 kV and 10 mA with a 0.5-mm copper and 1.0-mm Aluminium filter. The absorbed dose rate was measured at 0.722 $Gy \cdot min^{-1}$ at the abdomen of the guinea pigs.

Experimental protocol 1

Ascaris suum extract was prepared as previously described [12]. As shown in figure 1, 40 guinea pigs were injected intraperitoneally on days 1 and 14 with a mixture of 20 μg of *A. suum* protein and 20 mg of silica suspended in 1 mL of physiological saline. On day 21, the guinea pigs inhaled an aerosol of *A. suum* extract (1.25 $mg \cdot mL^{-1}$ in physiological saline) for 30 s through a face mask. The aerosol was generated with an ultrasonic nebulizer (NE-U06; Omron, Kyoto, Japan) and was administered at a constant flow of 10 $mL \cdot s^{-1}$. On day 25, 20 animals were irradiated at a dose of 8 Gy. On day 26, spleen cells (2×10^8) collected from 10 of the unirradiated guinea pigs were injected intravenously into 10 of the irradiated guinea pigs (designated the R-T group), and vehicle only was injected into the other 10 irradiated animals (designated the R group). The remaining 10 unirradiated guinea pigs were also injected with vehicle only and were designated the C group. On day 28, the guinea pigs in the R-T, R, and C groups were challenged first with an aerosol of physiological saline and then with an aerosol of *A. suum* extract (2.5 $mg \cdot mL^{-1}$ in physiological saline). The aerosols were directed at a constant airflow (10 $mL \cdot s^{-1}$) into a cylindrical adaptor (volume=10 mL) connected to the endotracheal tube. Each guinea pig inhaled the aerosol for 15 s. Baseline R_L was defined as the pulmonary resistance measured after saline aerosol inhalation. Subsequently, R_L was measured at 5, 10 and 15 min, and then on the hour, until 6 h after antigen inhalation. Airway secretions, if any, were removed with a paper string before each R_L measurement. Immediately after the last R_L measurement, the guinea pigs were deeply anaesthetized with an additional dose of urethane and killed by exsanguination from the abdominal aorta. Bronchoalveolar lavage (BAL) was then performed and was followed by lung fixation.

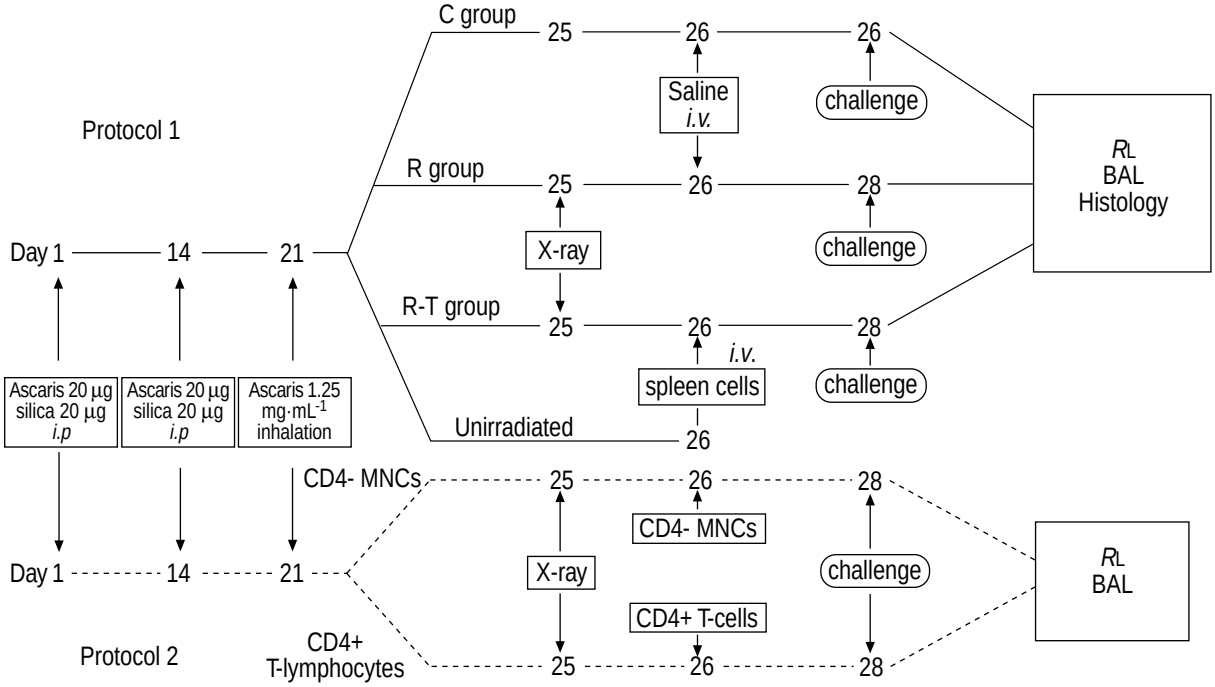

Fig. 1. – Flow diagram of protocol 1 (—) and 2 (- -). Numbers represent day number. C group (n=10) unirradiated guinea pigs injected with vehicle only; R group (n=10) irradiated guinea pigs injected with vehicle only; R-T group (n=10) irradiated guinea pigs injected with spleen cells from unirradiated guinea pigs. n=6 for CD4- MNCs and CD4+ T-lymphocytes; *i.p.*: intraperitoneally; MNCs: Mononuclear cells; R_L: pulmonary resistance; BAL: bronchoalveolar lavage.

On day 25 (before irradiation), day 26 (before spleen-cell or vehicle injection), day 27, and day 28 (before challenge), blood was collected from each animal's ear, and leukocytes in the sample were counted with a haemocytometer. Differential cell counts in blood smears stained with Wright-Giemsa were determined by counting >500 leukocytes at a magnification of $\times 1{,}000$ (oil immersion).

To examine the effect of the irradiation on total and differential cell counts in BAL and bone marrow, 15 additional guinea pigs were immunized in the manner described above. Five of the animals (the unchallenged-R group) were irradiated on day 25 and underwent BAL without antigen inhalation challenge on day 28. The other 10 animals (the unchallenged-C group) underwent BAL without irradiation or antigen inhalation challenge on day 28. After BAL, bone marrow was collected by the method of NANBA *et al.* [15] from all five animals in the unchallenged-R group and from half of those in the unchallenged-C group. Total and differential cell counts in bone marrow were determined as described above for peripheral blood samples.

Experimental protocol 2

To determine whether or not restoration of the LAR depends on the adoptive transfer of T cells, CD4+ T-lymphocytes were isolated from spleen cells of sensitized guinea pigs. Mononuclear cells (MNCs) were isolated from a crude spleen-cell preparation by density-gradient centrifugation on Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden). The isolated MNCs were resuspended in 2 mL of Hanks' balanced salt solution (HBSS; Gibco BRL, Rockville, MD, USA) with 2% foetal bovine serum (FBS; Gibco BRL). Thereafter, CD4+ T-lymphocytes were separated from CD4- MNCs by magnetic cell sorting (MACS), as described by MILTENYI *et al.* [16]. In brief, 10^6 MNCs were incubated first at 4°C for 15 min with 10 μL of mouse monoclonal antibody (mAb) to guinea pig CD4+ T-cells (Serotec Ltd., Oxford, UK) and then at 4°C for 15 min with microbeads directly conjugated with rat mAb to mouse IgG1 (Miltenyi Biotec, Auburn, CA, USA) (20 μL of magnetic microbeads per 10×10^6 cells). Labelled cells were subsequently removed by MACS and attached to a column in a magnetic field. After collection of negatively selected cells with an additional 20 mL of HBSS plus 2% FBS, the MACS column was removed from the magnet, and positively selected cells were eluted by flushing with 30 mL of HBSS plus 2% FBS. Consequently, $3–7 \times 10^6$ CD4+ T-lymphocytes and $20–30 \times 10^6$ CD4- MNCs were obtained from each sensitized guinea pig.

On day 26 (*i.e.* 24 h after whole-body irradiation of the immunized guinea pigs from protocol 1 at a dose of 8 Gy) CD4+ T-lymphocytes and CD4- MNCs were adoptively transferred to six animals each. On day 28, antigen inhalation challenge and BAL were performed as described above.

Bronchoalveolar lavage and lung fixation

The methods used for BAL and lung fixation have been described previously [13]. In brief, the lung was lavaged twice with 5 mL of physiological saline and then inflation-fixed with periodate-lysine paraformaldehyde *via* the trachea at 25 cmH_2O pressure. Total cell numbers in BAL fluid were counted with a haemocytometer. A 100-μL aliquot was cytocentrifuged with Cytospin 2 (Shandon, Inc., Pittsburgh, PA, USA). Differential cell counts were obtained from centrifuged preparations stained with Wright-Giemsa by counting >1,000 cells at a magnification of $\times 1{,}000$ (oil immersion). The fixed lungs were cut into sections 3 μm thick, stained with Diff-Quik solution I (Baxter, McGraw Park, IL, USA), and counterstained with haematoxylin. Three bronchioles were selected randomly from each section, and eosinophils (stained red) located in the bronchiolar wall (including smooth muscle and a cuff of 50 μm outside the muscle) were counted with a video micrometer (VM-30; Olympus, Tokyo, Japan) in a blind fashion.

Statistical analysis

The area under the percentage R_L curve (1 unit = $1\%{\cdot}h^{-1}$) 2–6 h after allergen inhalation was used for analysis of the LAR, while the maximal percentage change in R_L was used for analysis of the EAR. All results were expressed as mean±SEM values. Data obtained from more than three groups were analysed with the Dunn test after ANOVA. Data obtained from two groups were analysed with Welch's t-test. In all analyses, a p-value <0.05 was considered significant.

Results

Time course of pulmonary resistance

Since there were no significant differences in baseline R_L values among the C, R, and R-T groups (198.8±22.9, 189.0±14.5, and 151.0±9.6, respectively), changes in R_L in these three groups after antigen challenge are shown as percentages relative to each baseline value (fig. 2). In all three groups, an EAR was evident after

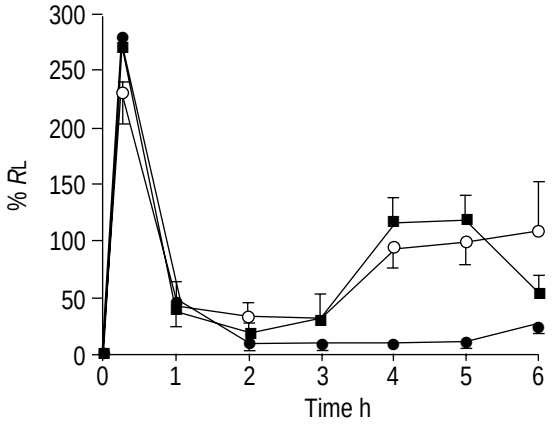

Fig. 2. – Time course for changes in pulmonary resistance (R_L) after Ascaris challenge in the C group (○), the R group (●), and the R-T group (■) (10 animals each; see text and fig. 1 legend for definition of groups)). Although there was no significant difference in %R_L at EAR among these three groups, %R_L at late asthmatic response was significantly smaller for the R group than for C and R-T groups. Values are expressed as mean±SEM.

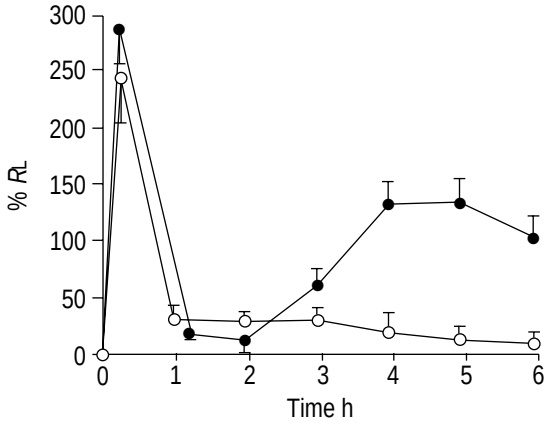

Fig. 3. – Time course for changes in pulmonary resistance (*R*L) after Ascaris challenge in animals that received either CD4+ T-lymphocytes (●) or CD4- mononuclear cells (MNCs) (○) (six animals each). Although there was no significant difference in %*R*L at early asthmatic response between the two groups, %*R*L at late asthmatic response was significantly greater for animals given CD4+ T-lymphocytes than for those given CD4- MNCs. Values are expressed as mean±SEM.

antigen inhalation, and *R*L returned approximately to the baseline value in the next hour. Thereafter, however, %*R*L for the R group did not increase at all, while %*R*L for the C and R-T groups gradually increased again 4–5 h after antigen inhalation, a change reflecting the LAR. Maximal increases in %*R*L during the EAR among the C, R, and R-T groups were 229.3±27.2, 278.7±40.2, and 270.6±46.5, respectively, and the area under the curve 2–6 h after allergen inhalation among the C, R, and R-T groups were 364.4±49.4, 62.8±10.4, and 334.4±66.8, respectively. Thus, whole-body irradiation at a dose of 8 Gy almost completely blocked the LAR without changing the EAR, and adoptive transfer of spleen cells from sensitized animals restored the LAR.

Figure 3 shows changes in *R*L after antigen challenge in guinea pigs that received either CD4+ T-lymphocytes or CD4- MNCs. A significant difference between groups was apparent in the LAR (439.4±62.1 *versus* 96.7±38.6), but not in the EAR (287.3±30.0 *versus* 244.7±38.2); specifically, a full LAR developed in animals given CD4+ T cells but not in those given CD4- MNCs. Thus, CD4+ T-lymphocytes were responsible for the appearance of the LAR in the guinea-pig asthma model.

Bronchoalveolar analysis

Differential cell counts in BAL fluid 6 h after antigen inhalation are presented in table 1. No significant difference was found between the C and R-T groups in terms of the total number of cells in BAL fluid. Although no difference in total cell count was evident among the unchallenged-C, unchallenged-R, and R groups, total cell numbers and eosinophil counts in these three groups were significantly lower than in the C and R-T groups. The lymphocyte count in the R group was significantly lower than that in the C and R-T groups. There were no significant differences among the five groups with regard to the number of neutrophils or macrophages. Thus, x-irradiation decreased eosinophil and lymphocyte counts in BAL fluid, and adoptive transfer of spleen cells restored these cell populations.

Table 2 shows differential cell counts 6 h after antigen inhalation in BAL fluid from guinea pigs that received either CD4+ T-lymphocytes or CD4- MNCs by adoptive transfer. The total cell count and the eosinophil count were significantly higher in guinea pigs given CD4+ T-lymphocytes than in those given CD4- MNCs, although macrophage and neutrophil counts were similar in the two groups. The lymphocyte count of the two groups differed, but not significantly. Thus, CD4+ T-lymphocytes in the transferred spleen-cell population played an essential role in the airway accumulation of eosinophils in the present model.

Histological analysis

Figure 4 illustrates the results of histological studies of guinea-pig lung tissues. Six hours after antigen challenge, eosinophil accumulation in bronchiolar walls was evident in an antigen-challenged, unirradiated animal (fig. 4a) but not in a challenged and irradiated animal (fig. 4b). The eosinophil response was restored by adoptive transfer of spleen cells from other

Table 1. – Differential cell counts in bronchoalveolar fluid from each of five groups of guinea pigs

Group	n	Total cells $\times 10^6$	Eosinophils $\times 10^6$	Macrophages $\times 10^6$	Neutrophils $\times 10^4$	Lymphocytes $\times 10^4$
C	10	22.7±2.3 (12.5–32.8)	15.9±1.9 (8.0–26.1)	6.0±1.0 (2.4–12.2)	56.0±24.3 (2–264)	15.7±3.4 (0–30)
Unchallenged-C	10	14.9±0.7$^{\#(*)}$ (11.9–18.4)	8.5±0.6***$^{(\#)}$ (6.2–11.3)	6.1±0.3 (4.5–7.4)	23.1±3.5 (4–40)	6.9±1.9 (0–16)
Unchallenged-R	5	14.3±2.0)$^{\#(*)}$ (9.3–20.8)	8.2±1.7***$^{(**)}$ (4.3–13.2)	6.0±1.0 (3.2–9.3)	19.8±7.5 (1–45)	2.4±1.0* (0-5)
R	10	14.6±1.5***$^{(**)}$ (7.0–21.2)	8.5±0.8***$^{(\#)}$ (3.7–12.7)	5.8±0.8 (2.4–9.7)	35.3±10.8 (0–90)	2.2±0.5**$^{(*)}$ (0–5)
R-T	10	20.7±1.4 (16.0–31.4)	14.4±1.2 (8.6–21.5)	5.8±0.8 2.9–10.8)	32.9±10.3 (4–105)	13.1±5.9 (0–64)

Values are expressed as mean±SEM (ranges) *: p<0.05; **: p<0.01; $^{\#}$: p<0.005; and ***: p<0.001 *versus* the C group or the (R-T group).

Table 2. – Differential cell counts in bronchoalveolar lavage fluid from guinea pigs received either CD4+ T-lymphocytes or CD4- mononuclear cells (MNCs) by adoptive transfer

Group	Total cells $\times 10^6$	Eosinophils $\times 10^6$	Macrophages $\times 10^6$	Neutrophils $\times 10^4$	Lymphocytes $\times 10^4$
CD4+ T cells	20.4±1.2 (16.6–25.3)	14.9±1.0 (11.6–18.2)	5.0±0.7 (2.7–6.6)	37.8±10.4 8–84	13.5±5.4 (0–39)
CD4- MNCs	14.1±2.4* (9.0–24.4)	9.0±1.3** (5.3–13.4)	4.7±1.1 (2.8–9.9)	35.3±17.1 (3–110)	2.0±1.1 (0–7)

Values are expressed as mean±SEM (ranges). There were six animals per group. *: $p<0.05$; and **: $p<0.01$.

immunized animals before challenge (fig. 4c). The numbers of eosinophils infiltrating the bronchiolar walls were significantly lower in the unchallenged-C (247±34), unchallenged-R (229±29), and R (337±161) groups than in the C (1057±179) and R-T (674±77) groups. Counts in the R-T and C groups did not differ significantly from each other.

Analysis of peripheral leukocytes and bone marrow

Figure 5 shows changes in peripheral-blood leukocyte counts from day 25 to day 28 (*i.e.* in the first 72 hours after irradiation) in the C, R, and R-T groups. The numbers of total cells, mononuclear cells, neutrophils, and eosinophils decreased significantly over this interval. Adoptive transfer of spleen cells did not alter these cell counts on the day of inhalation challenge.

Cell distributions in bone marrow obtained from irradiated and unirradiated guinea pigs are shown in table 3. Over 72 h, irradiation significantly decreased counts of total cells, erythroid cells, immature leukocytes, lymphocytes, and eosinophils but did not significantly affect numbers of neutrophils, plasma cells, or reticulum cells. Thus, irradiation significantly decreased numbers of lymphocytes and eosinophils not only in peripheral blood but also in bone marrow of guinea pigs.

Discussion

The present study examined the effect of whole-body x-irradiation on the EAR and the LAR in a guinea pig model of bronchial asthma. Whole-body irradiation almost completely blocked the R_L increase during the LAR but did not alter this increase during the EAR. In addition, irradiation not only inhibited the accumulation of eosinophils and lymphocytes in the airways 6 h after *A. suum* antigen challenge, but also significantly decreased the numbers of these cells in peripheral blood and bone marrow immediately before challenge. Adoptive transfer of spleen cells obtained from antigen-sensitized guinea pigs restored the increases in R_L during the LAR and the recruitment of eosinophils and lymphocytes into the airways. Further analyses revealed that the CD4+ T-lymphocytes from the transferred spleen-cell population were solely responsible for the appearance of the LAR and for cell infiltration of the airways. In short, it appeared that x-irradiation inhibited both the LAR and cell accumulation in the airways and that this effect was due to decreases in CD4+ T-lymphocyte counts.

In a preliminary study using doses of 2, 4, 8, and 12 Gy, the effect of x-irradiation on decreases in guinea-pig peripheral-blood leukocyte counts were evaluated. It was found that the number of leukocytes, especially MNCs, drastically decreased 3 days after all doses of irradiation, and that a dose of 8 Gy gave a submaximal effect. In 1955, ROSENTHAL [17] reported that myeloid and erythroid cells in bone marrow disappeared 3 days after irradiation with 6 Gy and that these cell populations did not regenerate thereafter in normal guinea pigs. In light of preliminary findings and the results reported by ROSENTHAL [17], 8 Gy was selected for the irradiation dose in the present study. Counts of MNCs, eosinophils, and neutrophils in peripheral blood significantly decreased during the 72 h after irradiation, and adoptive transfer of spleen cells did not alter these decreases at all. In addition, as expected, numbers of erythroid cells, immature leukocytes, lymphocytes, and eosinophils in bone marrow significantly decreased over this interval. No guinea pig died as a result of irradiation during this study.

To clarify whether sensitized lymphocytes can restore the LAR in this model, spleen cells from immunized guinea pigs were transfused into irradiated animals. Although cellular traffic was not defined after the transfer in this study, BERMAN *et al.* [18] have indicated that it takes 18–72 h for the distribution of lymphocytes to stabilize, and SCHUYLER *et al.* [19] have challenged animals 2 days after adoptive transfer in another model of cell-mediated pulmonary disease. Therefore, the presented protocol, in which animals were challenged 2 days after adoptive transfer, seems adequate to evaluate the function of transferred cells. This cellular supplementation almost completely restored the R_L increase during the LAR as well as the accumulation of eosinophils and lymphocytes in the airways. Because some investigators have reported that transfer of specific V beta T-cell subsets from sensitized mice increases airway responsiveness in naive recipients [20, 21] and that depletion of CD4+ T-lymphocytes completely prevents both antigen-induced airway hyperreactivity and recruitment of eosinophils in mice [22], the role of CD4+ T-lymphocytes separated from spleen cells of sensitized, unirradiated animals in the LAR and in airway infiltration by eosinophils was further examined. The transfer of CD4+ T-lymphocytes into irradiated animals restored the LAR and cell accumulation. Therefore, the authors suspected that CD4+ T-lymphocytes played a critical

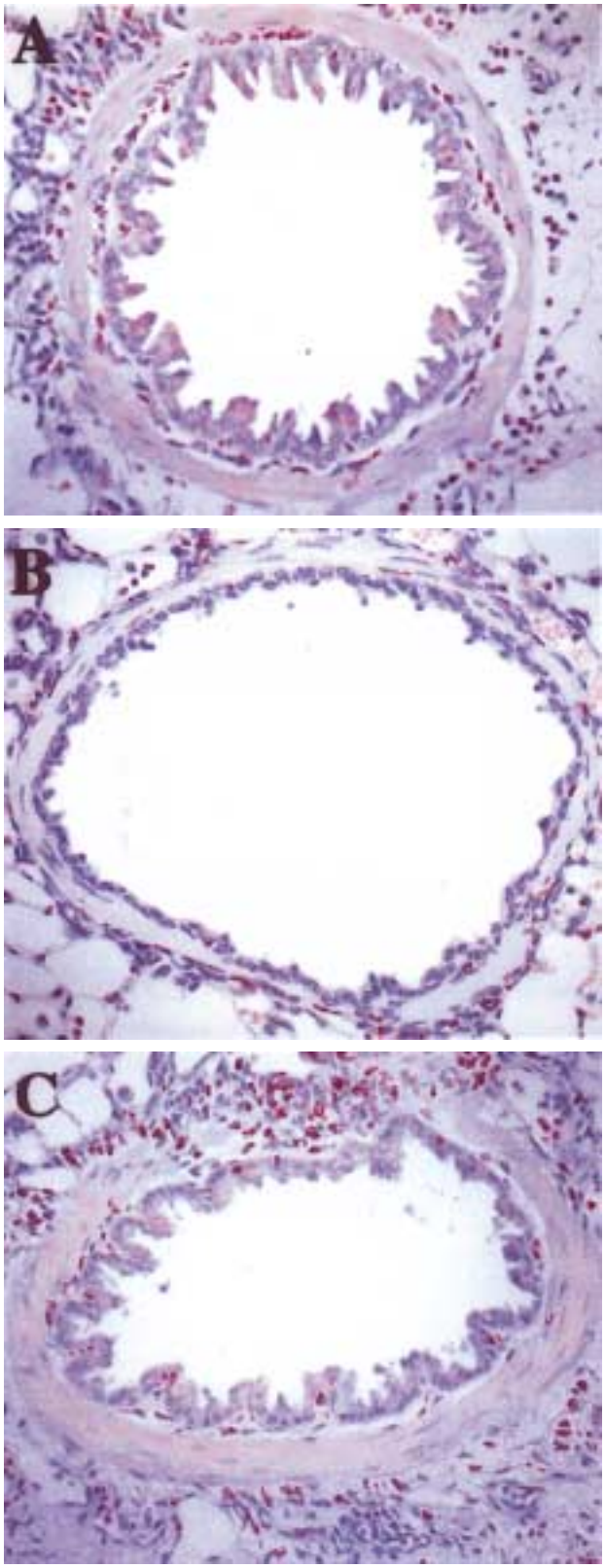

Fig. 4. – Photomicrographs of lung samples obtained from guinea pigs in a) the C group; b) the R group; and c) the R-T group (see text or fig. 3 legend for definition of groups), 6 h after antigen exposure. Remarkable eosinophil infiltration (stained red) is apparent in the animals from the C and R-T groups. The lung was stained with Diff Quik solution I and haematoxylin (original magnification; × 200).

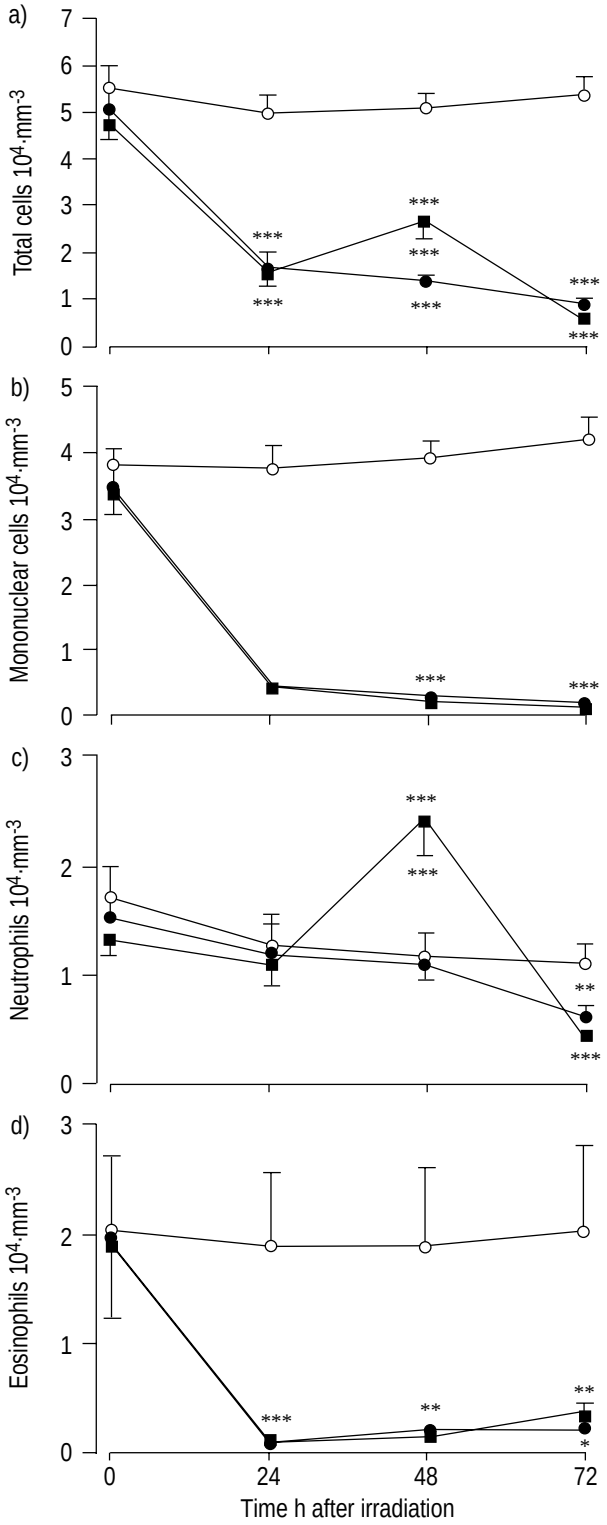

Fig. 5. – Changes in guinea-pig peripheral-blood leukocyte (a) total cells; c) neutrophils; b) mononuclear cells; and d) eosinophils) counts from day 25 to day 28, *i.e.* in the first 72 h after irradiation. There were 10 animals per group. Values are expressed as mean±SEM. Total cells, mononuclear cells, and eosinophils significantly decreased in number after x-irradiation (8 Gy); neutrophil counts decreased significantly only after 72 h. (○): c group; (●): R group; (■): R-T group; *: $p<0.05$, **: $p<0.01$, and ***: $p<0.005$ *versus* the C group at each time point. the (R-T group) at each time point. For definitions of groups see text or fig. 1 legend.

Table 3. – Differential cell counts in bone marrow from sensitized irradiated or unirradiated guinea pigs

Cell type	Unirradiated $\times 10^6$	Irradiated $\times 10^6$	p-value
Total cells	44.36±10.11	8.84±2.76	0.019
Erythroid cells	12.80±3.93	0.32±0.10	0.034
Immature leukocytes	7.56±1.31	0.38±0.15	0.006
Neutrophils	10.50±2.85	6.48±2.05	0.290
Eosinophils	5.06±1.42	0.98±0.34	0.049
Lymphocytes	7.64±2.14	0.00±0.00	0.023
Plasma cells	0.22±0.09	0.02±0.02	0.107
Reticulum cells	0.70±0.24	0.72±0.25	0.956

Values are expressed as mean±SEM. There were five animals per group.

role in the development of the LAR and in cell recruitment into airways in the guinea-pig asthma model.

Allergen exposure of irradiated animals 48 h after transfer of CD4+ T-lymphocytes resulted in a level of eosinophil recruitment into the airways as high as that in animals that were merely sensitized; however, irradiation with 8 Gy significantly decreased eosinophil counts in both peripheral blood and bone marrow immediately before challenge, and the transfer of spleen cells did not alter these decreases. In 1979, MULLER and MUNTENER [23] reported that irradiation caused extensive destruction of lymphocytes in the circulation and that this destruction was followed by the disappearance of eosinophils from the circulation and was paralleled by an increase in the number of eosinophils in the lymphatic organs. Still earlier, in 1953, CRONKITE [24] reported that mature eosinophils might be resistant to irradiation. Therefore, it is possible that the decrease in circulating eosinophils in the guinea pigs after irradiation may have been due to the redistribution of these cells into organs rather than to their destruction. In addition, it was found that small numbers of eosinophils remained in the bone marrow 3 days after irradiation. Since some investigators have reported a possible role for Th2 cytokines in late-phase lung inflammation in allergic mice, [25–27] it is believed that transferred CD4+ T-lymphocytes may have recruited eosinophils into the airways from the lymphatic organs and the bone marrow by releasing Th2 cytokines such as interleukins 4 and 5.

To conclude, in this model, x-irradiation abolishes the late asthmatic response, with significant inhibition of eosinophil infiltration into the airways after antigen inhalation challenge and this effect is due to the destruction of CD4+ T-lymphocytes. It is suggested that the late asthmatic response and cell recruitment into the airways are mediated by CD4+ T-lymphocytosis in the guinea-pig asthma model.

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