Influence of the thymus on the incidence of secondary and parabiotic disease

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Summary. The secondary disease in P → F_1 strain combination depends upon the immune status of both partners. It is most clearly expressed if both donors and recipients are immunologically crippled by thymectomy and irradiation. Conspicuous reduction in the immune potential of parental parabionts induced by thymectomy and irradiation displayed their marked incidence of parabiotic death.

One explanation of the pathogenesis of secondary disease emphasizes primarily the immunological incompetence and lymphoid deficiency[1-4], whereas an alternative explanation[5] attributes the disease also to a generalized graft-versus-host (GVH) immune reaction and the latent radiation damage. If the GVH reaction is primarily responsible for secondary disease, then thymectomy of donor and/or recipient mice may mitigate or delay the disease. If an immune deficiency leads to death, thymectomy could increase the mortality. Van Putten[6] reported a higher incidence of the secondary disease in thymectomized than in nonthymectomized syngeneic and P→F_1 radiation chimaeras. In contrast, the same report[6] demonstrated that thymectomized lethally irradiated mice reconstituted with xenogeneic bone marrow succumb later than nonthymectomized mice. Simmons et al.[7] showed that a secondary disease developed with equal rapidity in both thymectomized and sham thymectomized allogeneic chimaeras. Normal parental mice kill F_1 parabiotic partners, but parental strain parabionts suppressed by sublethal irradiation succumb when joined with their F_1 hybrids[8]. We investigated the effect of a T-cell deficiency on the incidence of the secondary and parabiotic disease.

Materials and methods. CBA, CBAT6T6, and (C57BL × CBAT6T6)F_1 mice of both sexes were used. Mice were thymectomized at 4–6 weeks of age, irradiated (900 R) at 12 weeks of age and within 3 h injected i.v. with 10^7 viable bone marrow cells. The procedures and irradiation constants have been described elsewere[9]. Mice were observed for a period of 120 days after irradiation, and reconstitution and mortality was scored daily. The method of cytogenetic analysis of T6 chromosome was used to determine the chimaerism[10]. The survival times between groups od radiation chimaeras were compared by Mann-Whitney modification of the U-test[11]. Irradiated and marrow-injected mice were used in parabiosis experiments 40 days later. Parabiosis was established by the methods of Bunster and Meyer (see Finerty[12]). Parabiotic pairs were observed daily until the death of 1 of the partners. After 1 partner died, the other was killed. The outcome of the parabiosis was analyzed by χ^2 test.

Results and discussion. Normal (nonthymectomized) F_1 mice were irradiated and reconstituted with bone marrow from normal (nonthymectomized) CBA mice or from thymectomized irradiated CBAT6T6 mice reconstituted with CBA bone marrow cells (TIR) thus producing conventional semiallogeneic radiation chimaeras IR and chimaeras IR_{TIR}. Thymectomized irradiated F_1 hybrids were reconstituted as above, nonthymectomized mice thus producing chimaeras TIR and TIR_{TIR}. Other similarly treated 4 groups were prepared with CBA mice as irradiated recipients.

Table 1 presents that semiallogeneic radiation chimaeras IR, whose lymphoid cells had been influenced by thymus in both, bone marrow donors and recipients, survived significantly longer than semiallogeneic radiation chimaeras IR_{TIR}, TIR and TIR_{TIR} with less thymus influence ($p < 0.05$ for each comparison). Semiallogeneic radiation chimaeras TIR_{TIR}, whose lymphoid cells had been influenced neither by donor nor by their own thymus, died significantly earlier than other semiallogeneic radiation chimaeras ($p < 0.05$ for each comparison). Survival times of semiallogeneic radiation chimaeras TIR and IR_{TIR} did not differ from each other. Survival times in the groups of syngeneic radiation chimaeras did not differ significantly from each other regardless of the treatment. They also showed no difference from semiallogeneic radiation chimaeras IR.In the chimaeras that survived for 120 days, only the cells of the donor karyo-

Table 1. Survival of thymectomized (T) or normal (C57BL × CBAT6T6)F_1 and CBA mice irradiated (I) with 900 R and reconstituted with 10^7 bone marrow cells from normal CBA (R) or TIR* CBA (R_{TIR}) mice

Bone marrow recipients	No. of mice	No. of surviving mice**	Median survival time (days)
F_1: IR	20	15	> 120
TIR	20	7	50
IR_{TIR}	20	3	36
TIR_{TIR}	20	0	25
CBA: IR	10	9	> 120
TIR	10	7	> 120
IR_{TIR}	10	7	> 120
TIR_{TIR}	10	5	> 120

* Thymectomized, lethally irradiated and reconstituted with syngeneic marrow cells. ** At 120 days after irradiation and bone marrow transfer.

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Table 2. Mortality of CBA and hybrid (C57BL × CBAT6T6)F_1 mice joined in parabiosis

Pretreatment of partners CBA	(C57BL × CBAT6T6)F_1	Number of pairs	Died (No. of days after parabiosis) (C57BL × CBAT6T6)F_1	Both	CBA
N	N[a]	15	15 (2×9, 10, 2×11, 2×15, 16, 17, 2×18, 19, 21, 22, 23)	0	0
	T[b]	15	15 (3×8, 2×9, 3×10, 11, 12, 13, 15, 17, 20, 21)	0	0
IR[c,d]	N	15	10 (11,12,15,17,18, 19, 21, 22, 24, 57)	5 (10, 14, 15, 18, 31)	0
	T	15	13 (10, 2×11, 12, 13, 3×14, 15, 17, 19, 24, 27)	2 (25, 28)	0
T	N	15	9 (9, 10, 12, 16, 20, 24, 26, 31, 32)	5 (6, 2×7, 9, 15)	1 (8)
	T	15	6 (18, 23, 30, 31, 35, 51)	9 (2×6, 2×7, 10, 13, 15, 17, 27)	0
TIR	N	15	0	14 (9, 16, 2×17, 2×21, 23, 24, 25, 27, 2×30, 31, 56)	1 (40)
	T	15	1 (25)	9 (10, 11, 12, 13, 2×14, 15, 20, 26)	5 (10, 19, 2×20, 46)

[a] Normal. [b] Thymectomized. [c] Irradiated (900 R). [d] Reconstituted with 10^7 syngeneic bone marrow cells.

type were found in spleen, lymph nodes, bone marrow and thymus when present. These results suggest that inadequate immunological protection plays in secondary disease a more important role than GVH reaction does. CBA mice used for parabiosis studies were normal N, irradiated and reconstituted with syngeneic bone marrow IR, thymectomized T and mice thymectomized irradiated and reconstituted with syngeneic bone marrow cells TIR. They were joined to normal N or thymectomized T F_1 hybrides of the same sex.

As shown in table 2, all hybrid partners succumbed in about 3 weeks when parabiosed with normal CBA parent. All hybrid partners were also killed when parabiosed to CBA IR mice, but some of the parental partners died on the same day, as did the hybrid mice. Mortality of parental partners was further increased by prior thymectomy. Finally, when parental CBA mice were both thymectomized and irradiated TIR, their mortality was greater than that of their hybrid partners. Also the highest frequency of deaths of both parabiotic partners was observed in groups in which the immune status of partners was impaired by thymectomy and/or irradiation. It appears that immunologically crippled parental partners succumbed due to a continuous immunological stimulus, which, exceeding the capacity of the immune system, led these partners to immunological exhaustion and consequent death[1,8].

Statistical analysis of IR, T or TIR CBA mice parabiosed with normal or thymectomized hybrid partners showed significant differences ($p < 0.001$ for each comparison). This suggests that the immunological status of hybrid partners also influences the outcome of the parabiosis.

Dexamethasone-induced neutrophilia. Negative correlation with increased plasma adrenaline concentrations

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Summary. Maximal increments in adrenaline and dexamethasone (DXM) plasma concentrations were observed c15 (T_{50} 40 min) and 30 (T_{50} 210–240 min) minutes after an i.v. DXM dose (6 mg/m² BSA) in man. There appears, however, to be no direct interaction between these agents in the development of induced neutrophilia, which occurs c240 min postinjection.

Glucocorticoids in general[2] and dexamethasone (DXM) in particular[3], induce a peripheral blood neutrophilia in man with conventional pharmacological dosage. The neutrophilia is maximally developed 4–6 h following either i.v. or oral administration and is associated with a profound lymphocytopenia[2]. It has been suggested that mobilization of the marginated granulocyte pool (MGP) may account for the increased circulating granulocyte pool (CGP), because of the presence during neutrophilia of cells with low alkaline phosphatase (AP) activity[3], presumably emerging from sequestered organ sites and

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