We also tested whether such effects could be seen in immunosuppressed mice. Mice were treated intraperitoneally with cyclophosphamide (200 mg/kg) on day 11, and GM-CSF was i.p. administered at various doses for 7 days before *C. albicans* infection. The anticandidal dose-response of i.p. GM-CSF (100 U/mouse) in immunosuppressed mice is shown in Fig. 2. As shown in Fig. 3, cyclophosphamide-treated mice died much earlier than did control mice. However, it was found that i.p. GM-CSF (100 U/mouse) could effectively protect against *C. albicans* infection even in immunosuppressed mice.

Effect of Cell Populations

We analyzed total white blood cell numbers and populations in peripheral blood after intraperitoneal GM-CSF administration to immunosuppressed mice. The time course of cell populations in peripheral blood of immunosuppressed mice after i.p. GM-CSF is shown in Table 1. Cyclophosphamide (200 mg/kg) was i.p. administrated on day 4 before i.p. GM-CSF (100 U/mouse).

Throughout the observation period from days 2 to 6, the percent ages of monocytes gradually increased with time, with the maximum increase observed on day 6, whereas the percent ages returned to normal on day 8. The lymphocyte percent ages gradually decreased after cyclophosphamide administration, whereas the percent age returned to normal on day 8. The PMN percent ages where apparently unaffected by cyclophosphamide. Furthermore, i.p. GM-CSF increased the percent age of PMN, whereas the percent age also returned to normal on day 8. We further found that the percent ages of macrophages in the peritoneal fluid increased with time after i.p. GM-CSF (100 U/mouse), whereas the percent age also returned to normal on day 8 (data not shown).

Effect of GM-CSF on NK Cell Activity

Effect of i.p. GM-CSF on NK cell activity was determined by radioactivity release assay from target YAC-1 cells according to the method described by Wright et al.[11] The percent lysis of YAC-1 cells by NK cells from normal mice was about 7.0% (Fig. 4). Although NK cell activity of cyclophosphamide-treated mice was lower than that of non-treated controls, the

Table 1. Effect of Intraperitoneally Administered GM-CSF on White Blood Cell Numbers and Percent Ages of Monocytes, Lymphocytes, and Polymorphonuclear Leukocytes in Immunosuppressed Mice[a]

Time after i.p. (d)	WBC numbers (mean ± S.D.)	Monocytes (%)	Lymphocytes (%)	PMN (%)
N	6090 ± 913	1.4 ± 0.3	60.7 ± 1.4	37.9 ± 0.8
2	1157 ± 185	1.5 ± 0.2	39.2 ± 1.5	59.3 ± 1.9
4	4894 ± 562	2.0 ± 0.2	43.8 ± 1.9	54.2 ± 1.1
6	5900 ± 598	2.6 ± 0.3	46.2 ± 1.8	51.8 ± 2.6
8	6251 ± 825	1.4 ± 0.3	58.9 ± 2.8	39.9 ± 1.8
Overall test *p*-value [b]	< 0.001	< 0.001	< 0.001	< 0.001
Multiple comparisons*	Day 8 > Day 2	Day 6 > Day N	Day N > Day 2	Day 2 > Day N
	Day 8 > Day 4	Day 6 > Day 2	Day N > Day 4	Day 2 > Day 4
	Day N > Day 2	Day 6 > Day 4	Day N > Day 6	Day 2 > Day 6
	Day N > Day 4	Day 6 > Day 8	Day 4 > Day 2	Day 2 > Day 8
	Day 6 > Day 2	Day 4 > Day N	Day 6 > Day 2	Day 4 > Day N
	Day 6 > Day 4	Day 4 > Day 2	Day 6 > Day 4	Day 4 > Day 6
	Day 4 > Day 2	Day 4 > Day 8	Day 8 > Day 2	Day 4 > Day 8
			Day 8 > Day 4	Day 6 > Day N
			Day 8 > Day 6	Day 6 > Day 8
				Day 8 > Day N

[a] Cyclophosphamide (200 mg/kg, single dose) was i.p. administered 4 days before i.p. GM-CSF administration. Data are expressed as the mean ± S.D. (n = 6). N: Normal control mice.
[b] Repeated measures ANOVA were used in overall tests.
* Statistically different at $\alpha = 0.05$.