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Cytokines in lethal graft-versus-host disease

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Graft-versus-host disease (GVHD) is caused by donor T lymphocytes that recognize foreign antigens on host tissues. This leads to T cell activation, which involves a cascade of events including the transcription of genes for cytokines and their receptors and the production of cytokines [1, 2]. One of the first cytokines to appear is interleukin 2 (IL-2). IL-2 production enhances the IL-2 receptor expression and leads to T cell proliferation. As a further step, differentation of T cells occurs, which results in the production of a certain pattern of cytokines. These cytokines influence the expression of cell surface antigens and adhesion molecules, and are able to activate other cell types such as cytotoxic T cells, macrophages and natural killer cells, which might act as effector cells in tissue destruction [2]. Insight into the sequential expression of the various cytokines involved might enable a more effective treatment of GVHD. Therefore, we investigated the occurrence of cytokines in a murine model for acute GVHD. We addressed in particular the period early after allogeneic reconstitution.

Key words: Graft-versus-host disease – Cytokines

Materials and methods

Mice. (C57BL/Ka × CBA/Rij)F1 ($H\text{-}2^{b/q}$) and BALB/c ($H\text{-}2^{d}$) mice were bred at the Department of Immunology of Erasmus University. The mice were 12–18 weeks old at the start of the experiments. Mice were kept two per cage in light-cycled rooms with access to acidified water and pelleted food ad libitum.

Determination of cytokines. Serum samples and supernatants from spleen cells cultured for 24 h with Con A (1 μg/ml) [3] were assayed for cytokine activity. IL-2 was determined by a proliferative assay using an IL-2 dependent CTLL cell line, maintained in vitro in medium supplemented with rhIL-2; we used rhIL-2 as a standard (gift from Sandoz Forschungsinstitut, Wien, Austria). For the detection of interleukin 6 (IL-6), the B9 cell line was used [4]; we used rmuIL-6 as a standard (British Biotechnology, Abingdon, UK). Tumor necrosis factor (TNF)-α levels were determined by a cytotoxicity assay on WEHI 164 cells [5]; we used rmuTNF-α as a standard (gift from BASF/Knoll, Ludwigshafen, Germany). The proliferative or cytotoxic activity was measured with the MTT assay [6]. Interferon (IFN)-γ was determined in a sandwich ELISA, using a rat-anti-mouse IFN-γmAb (XMG1.2) as a catching antibody and a polyclonal rabbit-anti-mouse IFN-γAb as a second step. We used rmulFN-γ purified from CHO cells transfected with the mulFN-γ gene as a standard (kind gift from Dr. R. L. Coffman, DNAX Research Institute, Palo Alto, Calif).

Other procedures. Preparation of cell suspensions, induction of GVHD, collection of serum samples and data analysis were performed as described by us in earlier studies [7, 8].

Results and discussion

Serum samples obtained from lethally irradiated (C57BL × CBA)F1 mice, in which a lethal graft-versus-host reaction was induced by injection of 10^7 allogeneic BALB/c spleen cells, were analyzed for cytokine activity. As a control, serum samples from similarly treated mice injected with 10^7 syngeneic spleen cells were used. The results are summarized in Table 1. Since mortality occurred at day 8, from that day on the number of mice in each group became too small for statistic analysis. Therefore, no data are presented after day 8. It appeared that IL-6 levels increased from day 4 in the allogeneically reconstituted mice with a peak level at day 8, in contrast to syngeneically reconstituted mice in which no rise was found. Serum IFN-γ levels increased strongly in the allogeneically reconstituted mice between day 4 and 5, reaching a peak level at day 6. No rise was seen in the syngeneically reconstituted mice. We further determined the TNF-α activity in sera from both groups of mice. Signi-

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Table 1. Symptoms of GVHD in relation to serum cytokine levels after allogeneic and syngeneic reconstitution of lethally irradiated mice. (C57BL × CBA)F1 mice were lethally irradiated and reconstituted either with 10^7 allogeneic BALB/c spleen cells or with 10^7 syngeneic spleen cells. At various days after reconstitution, serum samples were obtained from mice of each group, and analyzed for cytokine activity. Furthermore clinical symptoms were evaluated in both groups of mice

	Days after reconstitution					
	3	4	5	6	7	8
Symptoms of disease[a]	−	−	−	+	+ +	+ + +
IL-2	−	−	−	−	−	−
L-6	−	+	+	+ +	+ +	+ + +
IFN-γ	−	−	+ +	+ + +	+ +	+ +
TNF-α	−	n.d.	−	n.d.	+ + +	n.d.
Symptoms of disease[b]	−	−	−	−	−	−
IL-2	−	−	−	−	−	−
IL-6	−	−	−	−	−	−
IFN-γ	−	−	−	−	−	−
TNF-α	−	n.d.	−	n.d.	+	n.d.

[a] after allogeneic reconstution
[b] after syngeneic reconstitution
− not detectable; + low/light; + + moderate; + + + high/severe; n.d. not determined

ficantly increased TNF-α levels were found at day 7 after allogeneic reconstitution. Serum IL-2 levels were below the detection limit (0.1 U/ml).

Since the detection of cytokines in the serum might be hampered by inhibitory or binding factors, e.g. soluble receptors, we further analyzed tissue culture supernatants of spleen cells 24 h after culture in the presence of Con A. A significantly higher activity of both IL-6 and IFN-γ was found in spleen cell supernatants from allogeneically reconstituted mice as compared to syngeneically reconstituted mice, showing a similar time-dependence as the serum levels. The TNF-α activity in spleen cell cultures was very low in both groups. IL-2 levels were below the detection limit.

Evaluation of clinical symptoms in both groups revealed that in the allogeneically reconstituted mice symptoms of acute GVHD were present at about day 6. Mortality in these mice occurred between 8 and 24 days after reconstitution, with a mean survival time of 12.5 ± 4.9 days. In the syngeneically reconstituted mice no signs of disease were found (Table 1).

Taken together, the above data indicated that a rise in IFN-γ and IL-6 levels, detectable both in serum and culture supernatants preceded the clinical symptoms and mortaility in acute GVHD. The clinical symptoms of GVHD seemed to be most closely associated with a rise in serum TNF-α activity. It is likely that the expression of these cytokines was preceded by cytokines the appeared earlier, especially IL-2. Although we could not detect IL-2 activity, possibly due to the fact that IL-2 is rapidly consumed, an important role for IL-2 was suggested by preliminary data showing decreased morbidity and mortality in acute GVHD after the in vivo administration of anti-IL-2 or anti-IL-2 receptor mAb. This stresses the importance of the use of complementary assay systems to determine the role of cytokines. Studies are underway to determine whether other cytokines also play a role in this complex disease.

References

1. Krensky AM, Weiss A, Crabtree G, Davis MM, Parham P (1990) N Engl J Med 322: 510
2. Ferrara JLM, Deeg JH (1991) N Engl J Med 324: 667
3. Cleveland MG, Annable CR, Klimpel GR (1988) J Immunol 141: 3349
4. Aarden LA, Groot de ER, Schaap OL, Lansdorp PM (1987) Eur J Immunol 17: 1411
5. Meager A, Leung H, Woolley J (1989) J Immunol Methods 116: 1
6. Mosmann T (1983) J Immunol Methods 65: 55
7. Knulst AC, Bril-Bazuin C, Benner R (1991) Eur J Immunol 21: 103
8. Knulst AC, Bazuin C, Benner R (1989) Transplantation 48: 829