American Journal of Pathology, Vol. 151, No. 3, September 1997

Defective Development of Pristane-Oil-Induced Plasmacytomas in Interleukin-6-Deficient BALB/c Mice

Giuseppe Lattanzio,* Claude Libert,† Massimo Aquilina,† Manuela Cappelletti,† Gennaro Ciliberto,† Piero Musiani,* and Valeria Poli†

From the Dipartimento di Patologia Umana e Medicina Sociale, Università di Chieti, Chieti, and the Istituto di Ricerche di Biologia Molecolare P. Angeletti,† Pomezia, Rome, Italy*

Interleukin (IL)-6 is known to be an essential growth factor for myeloma cells, both *in vitro* and *in vivo*. In mice, IL-6 is required for development of B cell tumors upon infection with a retrovirus expressing the *myc/raf* oncogenes. In the present study, we used the pristane-oil-induced plasmacytoma model, which more closely mimics tumor transformation and progression in human multiple myeloma. Also using this system, we found that IL-6-deficient BALB/c mice are protected against tumor development. Although the pristane-induced inflammatory reaction was less pronounced in IL-6-deficient mice *versus* their wild-type littermates, both B cell differentiation and plasma cell formation took place, and even morphological evidence of plasma cell transformation was detected, albeit at a low frequency. However, in the absence of IL-6, there were never signs of uncontrolled proliferation of either normal B lymphocytes or tumor cells, suggesting that the role of IL-6 in murine plasmacytoma and possibly also in human multiple myeloma is to ensure abnormal survival and proliferation of previously transformed tumor cells and therefore tumor development and progression. *(Am J Pathol 1997, 151:689–696)*

Interleukin (IL)-6 is a multifunctional cytokine that plays a role in various aspects of immune response, hemopoiesis, and inflammation (reviewed in Ref. 1), and its dysregulated production has been implicated in the pathogenesis of several diseases including autoimmune disorders, postmenopausal osteoporosis, and plasma cell dyscrasias.[2–4] Indeed, IL-6 is known to be an essential growth factor for human multiple myeloma (MM) cells. Myeloma-derived cells and cell lines are dependent on IL-6 for their growth,[5–7] and MM patients have high circulating levels of this cytokine.[8–10] Moreover, treatment with IL-6-neutralizing monoclonal antibodies can improve several disease parameters in late-stage patients.[11,12] Malignant plasma cells carry somatic mutations in their Ig genes, implying that the tumor stem cell originated from plasmablastic cells, which are generated from centrocytic B cells upon antigen stimulation in peripheral lymphoid organs (reviewed in Ref. 13). Although IL-6 is not believed to be important in mediating the differentiation of centrocytic cells into plasmablastic cells, it is the only proliferation factor for early plasmablastic cells identified so far and is also a potent inducer of the development of proliferating plasmablastic cells into mature high IgG-producing nondividing plasma cells.

IL-6 has been shown to play a primary role in the development of B-cell neoplasias also in mice. This cytokine is an essential factor for the *in vitro* growth of plasmacytoma cells,[14–16] and IL-6 transgenic mice crossed with the BALB/c strain spontaneously develop plasmacytomas carrying a translocation of the c-*myc* gene.[17] The importance of IL-6 in the development of B-cell neoplasias has been recently demonstrated by Rudikoff and collaborators,[18] who have shown that IL-6-deficient mice are protected against the development of the plasma cell, but not of the myeloid cell, tumors induced by infection with a *myc/raf*-expressing retrovirus. However, these results do not clarify whether IL-6 is exerting its effect at the level of tumor onset, of its development, or both. A model that allows the study of B-cell tumor onset and progression without introducing transforming oncogenes is the analysis of plasmacytomas elicited in mice of the BALB/c strain by means of intraperitoneal injections with pristane oil (reviewed in Ref. 19). Remarkably, although *myc/raf*-induced tumors predominantly express IgM,[20] indicating that the tumor transformation involves early B cells, in analogy with what happens in human MM, pristane-induced plasmacytoma cells are predominantly IgA or IgG secreting and are therefore derived from B cells that underwent antigen stimulation. To gain further insights into the role of IL-6 in the onset and development of pristane-induced plasma cell tumors, we have introduced a null mutation of the IL-6 gene obtained by gene targeting into the BALB/c genetic

Accepted for publication May 27, 1997.

Dr. Libert's present address: Laboratory for Molecular Biology, Gent, Belgium.

Address reprint requests to Dr. Valeria Poli, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, UK.

background. We show here that granuloma formation and B cell activation occurred in the BALB/c IL-6-deficient mice, although at a lower level than in the wild-type counterparts, and that we could occasionally even spot plasma cells with anaplastic features. However, the IL-6-deficient mice never developed clinically or histologically diagnosable plasmacytomas, thus highlighting the crucial pathogenic role played by this cytokine in the progression and maintenance of B-cell neoplasias.

Materials and Methods

Mice and Treatments

The IL-6 mutant allele was introduced into the Balb/cAn genetic background through five consecutive backcrosses between IL-6+/− mice[3] and Balb/cAn mice (obtained from Charles River, Italy). For the first of such backcrosses, a Balb/cAn male was used to transmit the BALB/c Y chromosome, and subsequently males carrying the IL-6 null allele were selected and mated with Balb/cAn females. F5 mice heterozygous for the IL-6 null allele were intercrossed to generate BALB/c IL-6−/− or +/+ mice, which were then used for the experiments. The genotype of the mice with respect to the IL-6 allele was determined by Southern blot as previously described.[3] The 6-week-old mice were transferred from the Istituto di Richerche di Biologia Molecolare specific-pathogen-free facility to a conventional facility, and treatments with pristane oil were started 4 weeks later. Mice were injected intraperitoneally three times with 500 μl of pristane oil (2,6,10,14-tetramethyl-pentadecane, Sigma Chemical Co., St. Louis, MO) at 60-day intervals as described.[21] Procedures involving animals and their care were conducted in conformity with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana n. 40, February 18, 1992; NIH Guide for the Care and Use of Laboratory Animals, NIH publication 85–23, 1985).

Analysis of Ascitic Fluids

Starting 134 days after the initial injection of pristane, mice were examined for ascites appearance, and each mouse developing ascites underwent paracentesis. Ascitic fluids were diluted 1:10 in 10% formaldehyde, and 500 μl was cytocentrifuged (Cytospin 2, Shandon Scientific, Runcorn, UK) at 1250 rpm (500 × *g*) for 5 minutes. The air-dried smears were stained with diluted Giemsa solution (0.15 mol/L phosphate buffer, pH 6.6; Giemsa, 10:1) for 10 minutes and observed for cellular content. The mice tolerated paracentesis very well, and this procedure was repeated every 14 days until day 275 after the first treatment.

Histology and Immunochemistry

Animals were sacrificed by cervical dislocation. For histological evaluation, tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) or Giemsa. For immunohistochemistry, all sections after deparaffination were irradiated twice for 5 minutes in citrate buffer using a microwave oven (output, 500 W) for the reactivation of antigenic determinants inactivated by formalin fixation. Sections were then incubated with anti-mouse kappa or lambda light chain rat monoclonal antibodies (PharMingen, San Diego, CA), washed, and overlaid with biotinylated rabbit anti-rat Ig (Vector Laboratories, Burlingame, CA). Unbound antibodies were removed by washing, and the slides were incubated with Strep-AB complex/horseradish peroxidase (Dako, Milan, Italy).

Statistical Analysis

Statistical analysis on the data was performed using the analysis of variance test with the Statview computer program.

Results

Development of Ascites Is Delayed and Reduced in BalbC/IL-6−/− Mice

As the genetic background is known to be important for plasmacytoma development,[22] we have generated Balb/cAn/IL-6 (BIL-6) −/− and +/+ mice by intercrossing mice heterozygous for the mutation obtained through five consecutive backcrosses with mice of the Balb/cAn strain. BIL-6−/− and +/+ mice together with wild-type Balb/cAn (WT) mice as controls were injected intraperitoneally with pristane oil three times with a time interval of 60 days. Scoring for ascites appearance started 2 weeks after the last injection (day 134 after the first day of treatment) and was continued up to day 275, when all mice were sacrificed and tissues were analyzed histologically. Ascites first developed in both BIL-6+/+ and WT mice. Although both the volume of ascitic fluid and the time of its appearance were somewhat variable between individuals, all BIL-6+/+ and WT mice developed ascites during the observation period, and most of them started to show the distinctive abdominal swelling from day 134 after the first injection of pristane. In contrast, ascites development was delayed by 6 weeks in the BIL-6−/− mice, as it was only observed starting from day 177. In addition, with the exception of only one mouse, the amount of ascitic fluid as judged by the degree of abdominal swelling never reached the highest degrees observed in the BIL-6+/+ or WT mice, and 4 of 11 BIL-6−/− mice never developed a detectable ascites at all. Figure 1 shows the temporal pattern of ascites formation and its degree in BIL-6+/+ and −/− mice. It is worthwhile noting that the differences in ascites degree among the two groups of mice were always found to be statistically significant (from $P < 0.0001$ to $P < 0.03$), with the only exception of day 191, in which the difference was borderline ($P = 0.0564$).

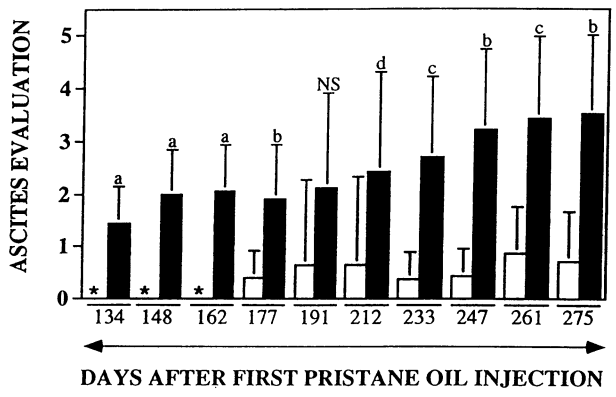

Figure 1. Ascites onset and evaluation in BIL-6+/+ (■) and −/− (□) mice between days 134 and 275 after the first pristane injection. Based on the degree of abdominal swelling, a 1 to 5 arbitrary score was assigned to each mouse. Mean scores + SD are indicated. There were 11 BIL-6−/− mice and 18 BIL-6+/+ mice. Letters over the histograms represent the statistical significance (P value) of the difference between −/− and +/+ mice: a, $P < 0.0001$; b, $P < 0.001$; c, $P < 0.002$; d, $P < 0.03$; NS, not significant ($P = 0.0564$). *No ascites.

Cellular Composition of the Ascitic Fluids and Plasmacytoma Development

Every mouse showing abdominal swelling underwent paracentesis every two weeks. Cytocentrifuged ascitic fluids were stained and analyzed for cellular content. The differential counts (percentage ± SD) of granulocytes, lymphocytes, and histiocytes present in the various ascitic samples obtained from BIL-6+/+ and −/− mice are shown in Figure 2a.

The cellular composition of the ascitic fluid obtained from BIL-6+/+ mice between day 134 (corresponding to the time of first ascites appearance) and day 162 after the first injection of pristane was mainly represented by granulocytes (~50%) and lymphocytes (~40%). At day 162, the granulocyte percentage started to sharply decrease, falling below 10% from day 220 on. At the same time, the lymphocyte percentage began to increase, reaching 65 to 70%, and the histiocyte content, initially below 5%, also increased, reaching 25 to 30% after 232 to 275 days. In parallel with these dramatic changes in cellular composition, increasing numbers of mature and often atypical plasma cells were detected in the cytocentrifuged samples, reaching 4 to 5% of the total cell content by day 205 (Figures 2b and 3, a and b). Plasmacytomas were diagnosed by the presence of at least 3% plasma cells in the cytocentrifuge smears of ascitic fluids, and the diagnosis was confirmed by postmortem examination and histological analysis. The first diagnosis of plasmacytoma based on the analysis of the ascitic fluids was not confirmed in only 2 cases of 18 examined. A total of 53.6% of the BIL-6+/+ mice and 51% of the WT mice developed the tumor. Interestingly, although the presence of ascites seemed to be a requirement for tumor development and its degree correlated well with the extent of the inflammatory process, there was no correlation between ascites degree and plasmacytoma development. No differences were observed between males and females within the three groups of mice.

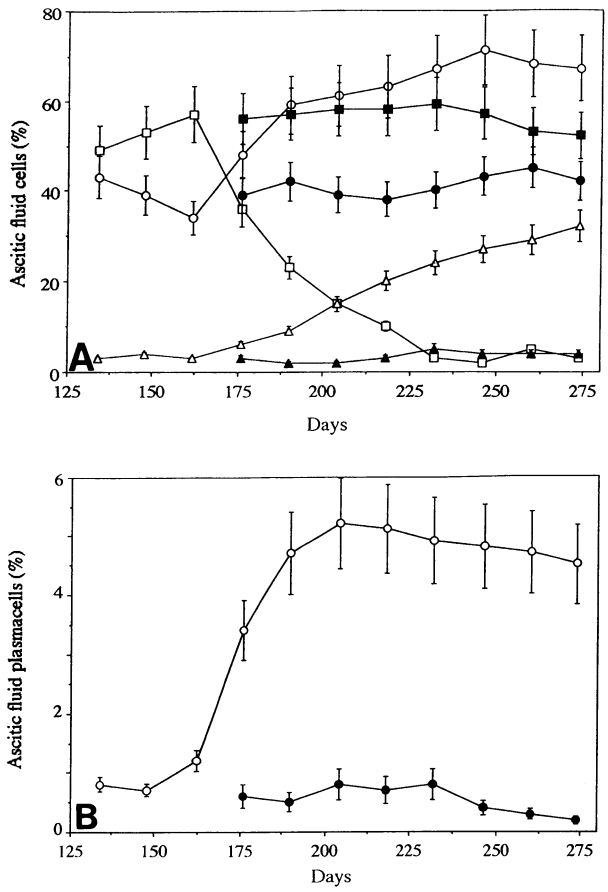

Figure 2. Differential cell counts (% ± SD) on the cytocentrifuged ascitic fluids from BIL-6+/+ (□, ○, and △) and BIL-6−/− (■, ●, and ▲) mice between days 134 and 275 after the first injection of pristane. Cell counts could start only from day 175 in the BIL-6−/− mice as no mouse developed ascites before that day. **A:** Granulocytes (□ and ■), lymphocytes (○ and ●), and histiocytes (△ and ▲). **B:** Plasma cells (○ and ●).

The analysis of ascitic fluids from the BIL-6−/− mice could be started only at day 177, due to the delay in their appearance. The cellular composition of their ascitic fluids did not differ from the initial composition of those from the BIL-6+/+ or the WT mice, being mainly formed by granulocytes and lymphocytes (Figures 2a and 3, a and d). Remarkably, however, and in contrast with what we observed in the case of the BIL-6+/+ mice, the levels of these two cell subsets remained constant throughout the whole observation period. Moreover, the histiocyte number was constantly very low (Figure 2a), and the plasma cell level never reached 1% (Figure 2b). As a consequence, plasmacytomas were never diagnosed in the BIL-6−/− mice, and this observation was confirmed by the postmortem histological analysis.

Histological Analysis of the Mesenteric Tissue Shows a Lower Degree of Inflammation and No Plasma Cell Tumor in the BIL-6−/− Mice

The histological analysis of mesenteric tissue from a BIL-6+/+ mouse sacrificed at day 162 (2 weeks before the dramatic changes in cellular composition were observed

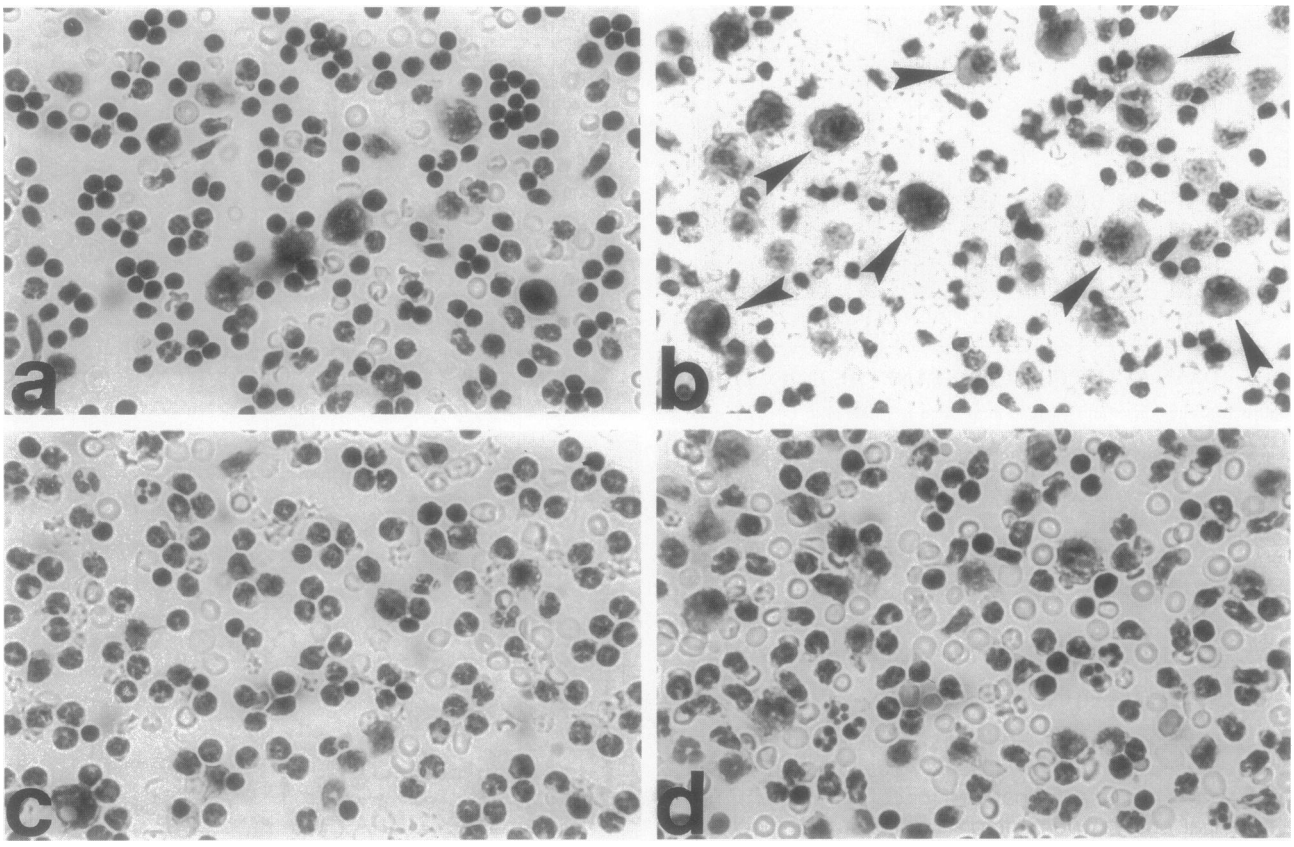

Figure 3. Cytocentrifuged smears of ascitic fluids obtained from BIL-6+/+ (**a** and **b**) and −/− (**c** and **d**) mice. Magnification, ×630. **a** and **b**: BIL-6+/+ mice on day 148 (**a**) and day 232 (**b**) after the first injection of pristane. The cell content is formed mainly by granulocytes and lymphocytes. Some mature and sometimes atypical plasma cells (**arrowheads**) are present in **b** (note the asymmetric position of the nucleus and the pale paranuclear area in the dark cytoplasm). **c** and **d**: BIL-6−/− mice on day 176 (**c**) and day 232 (**d**) after the first injection of pristane. The cell content is formed mainly by granulocytes and lymphocytes in both **c** and **d**.

in the ascitic fluid) showed the presence of granulocytes and activated histiocytes surrounding pristane transparent oil droplets (Figure 4a). Mesenteric fibroblasts showed morphological features of activation. Numerous small lymphocytes were present in the proximity of B-cell blast clusters. Foci of mature and/or atypical plasma cells were placed in the fibroadipose tissue under the peritoneal mesothelium. Figure 4b shows the histological analysis of a mouse sacrificed at day 232 after a clear cytological diagnosis of tumor, where a large part of the mesenteric tissue was replaced by plasmacytoma cells. The same picture was observed in more than 50% of the BIL-6+/+ mice sacrificed at the end of the observation period (day 290). Interestingly, in the mice that did not develop the tumor, the peritoneal inflammatory process was at this point much less severe than that observed in the mice sacrificed at day 160. In these mice, the mesenteric cellular infiltrate was mainly formed by several lymphocytes with some mature plasma cells. Histiocytes surrounding several oil droplets were still present, but they presented much less pronounced characteristics of activation (data not shown).

In the BIL-6−/− mice, the inflammatory process occurring around pristane droplets was less remarkable than in the BIL-6+/+ or in the WT mice. Numerous granulocytes and lymphocytes were present as well as histiocytes and fibroblasts even when in a scant number and more rarely showing morphological features of activation (Figure 4, c and d). From days 162 to 204, the mesenteric tissue presented several aggregates of B-cell blasts with mature plasma cells. Afterwards, the B-cell blast number remarkably decreased, and just some granulocytes and lymphocytes with several plasma cells could be found (Figure 4d).

Detection of Atypical Plasma Cells in BIL-6−/− Mice

Although plasmacytomas were never diagnosed in BIL-6−/−mice based on the low percentage of plasma cells scored in the ascitic fluids, plasma cells with atypical features were spotted in the cytocentrifuge smears of two of the six mice that developed ascites. In addition, serial histological studies on the mesenteric tissue obtained from one of these two BIL-6−/− mice, which was sacrificed immediately after the detection of the atypical plasma cells (day 200), showed the presence of blast cell aggregates somewhat resembling the germinal centers of the lymph node follicles. Centroblast-like cells with irregular nuclei and prominent nucleoli were present (Figure 5a), and near the mesenteric surface, few foci of

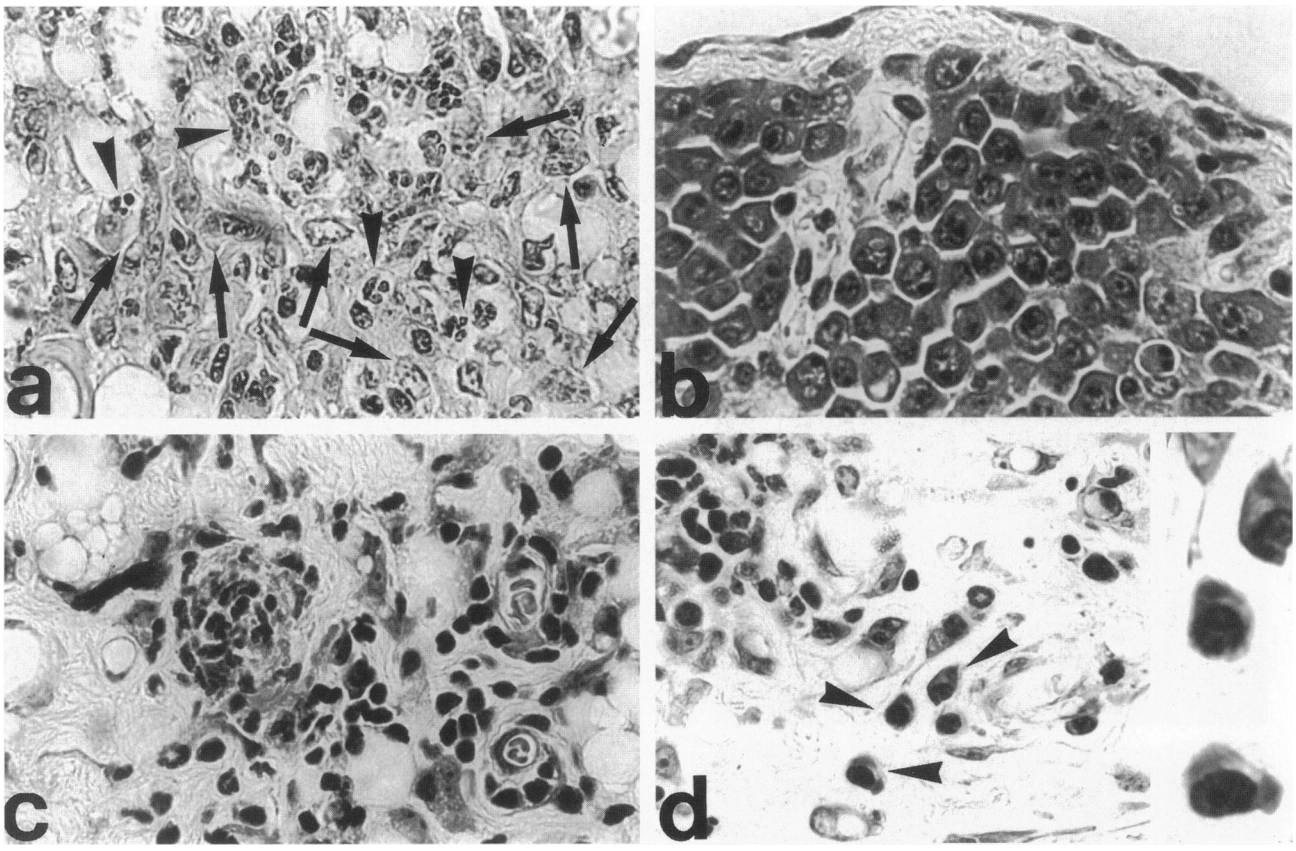

Figure 4. Histological analysis of mesenteric tissues obtained from BIL-6+/+ (**a** and **b**) and −/− (**c** and **d**) mice sacrificed at different times after the first injection of pristane. Magnification, ×630. **a:** Day 162. Several granulocytes (**arrowheads**) and activated histiocytes and fibroblasts (**arrows**) are present in the granulation tissue. Oil droplets are visible. **b:** Day 232. The mesenteric tissue is markedly infiltrated and replaced by plasmacytoma cells. **c:** Day 162. **d:** Day 232. Granulocytes and lymphocytes are present in both **c** and **d** among numerous oil droplets; some mature plasma cells (**arrowheads**) are observed in **d** and shown at higher magnification in the **inset** (×1000).

mostly mature plasma cells were identified, some of which displayed atypical or anaplastic features (Figure 5b). Immunohistochemical analysis showed that these atypical cells stained intensely for kappa light chains (Figure 6a) whereas they were negative for lambda light chains (not shown), thus confirming their plasma cell nature. This pattern was very similar to that shown by BIL-6+/+ mice with plasmacytoma, also displaying abnormally big plasma cells that specifically stained for kappa but not lambda light chains (Figure 6c and not shown). In contrast, immunohistochemical staining of mesenteric tissues from BIL-6+/+ mice not affected by

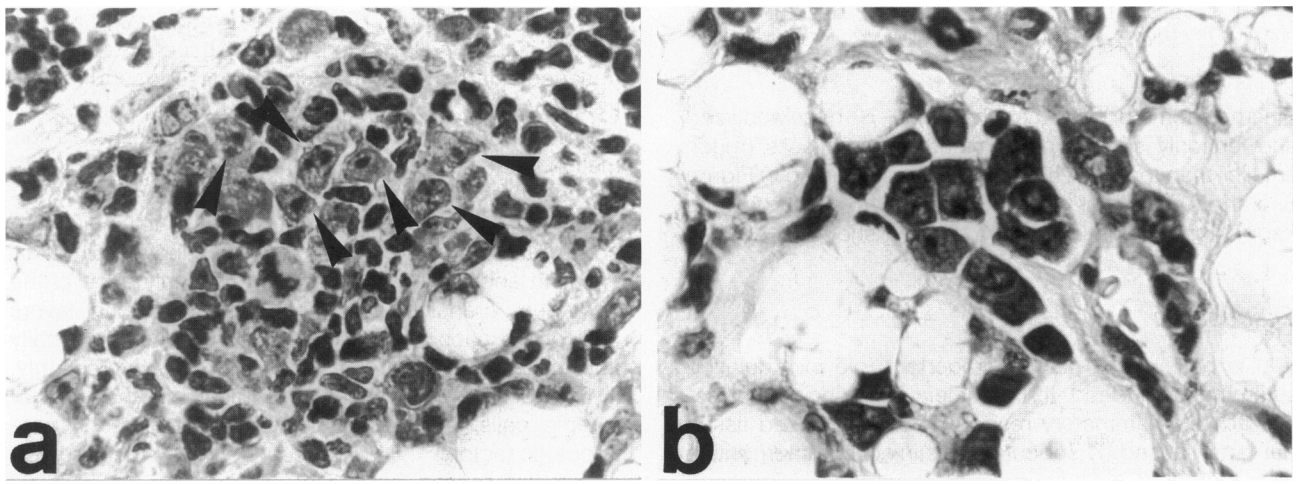

Figure 5. Mesenteric tissue obtained from one BIL-6−/− mouse 200 days after the first injection of pristane. Magnification, ×630. **a:** A blast cell aggregate somewhat resembling the germinal center of the lymph node follicle is shown. Centroblast-like cells (**arrowheads**), some with irregular nuclei and prominent nucleoli, are present. **b:** Aggregates of plasma cell precursors, some with atypical and anaplastic features.

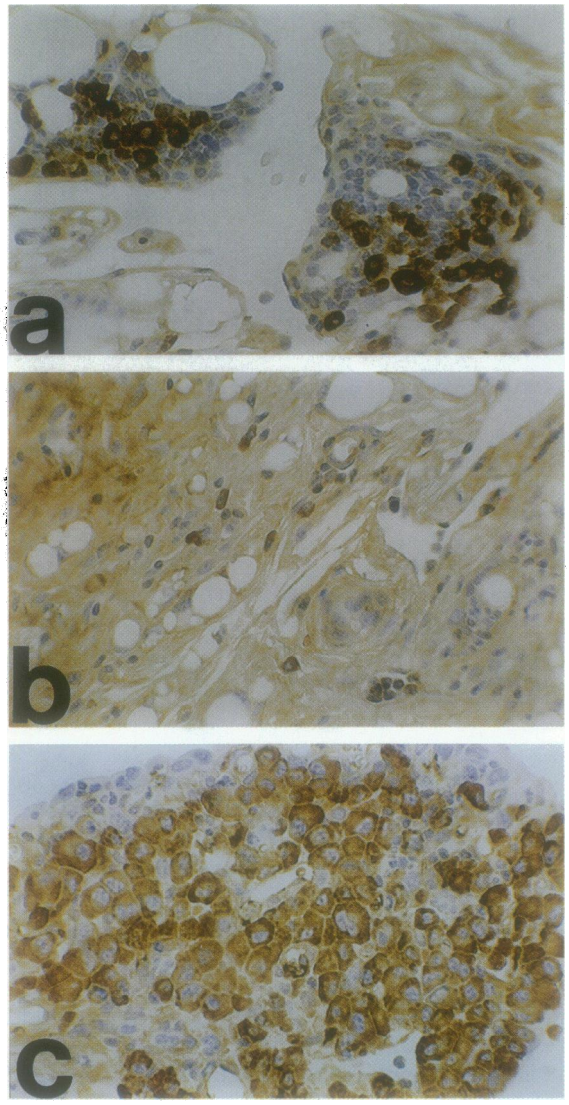

Figure 6. Mesenteric tissue obtained from BIL-6−/− (**a** and **b**) and +/+ (**c**) mice. Magnification, ×630. **a:** BIL-6−/− mouse 200 days after the first injection of pristane. Several large centroblast-like cells and plasma cell aggregates stain intensely for kappa light chains. **b:** BIL-6−/− mouse 232 days after the first injection of pristane. Some mature plasma cells are stained for light chains. **c:** BIL-6+/+ mouse 232 days after the first injection of pristane. The plasmacytoma cells are intensely stained for kappa light chains.

plasmacytoma or from the other BIL6−/− mice analyzed showed only some typical mature plasma cells (much smaller than those identified by immunostaining in Figure 6, a and c), some of which expressed lambda and some kappa light chains (Figure 6b and not shown).

Discussion

IL-6 is believed to play an important role in regulating inflammation. Indeed, IL-6-deficient mice showed defective acute inflammatory responses to a localized tissue damage caused by subcutaneous injection of turpentine oil.[23] Moreover, we have recently observed that impaired leukocyte accumulation occurred in the absence of IL-6 in response to carrageenan injected in subcutaneous air pouches.[24] In light of these findings, it is perhaps not surprising that the overall inflammatory process induced by pristane oil in the BIL-6−/− mice was less pronounced than in the BIL-6+/+ controls. Ascitic fluids were delayed in their appearance and constantly quantitatively scarce, and although the characteristic granulomas did appear in the peritoneum, histiocytes and fibroblasts were less abundant in the granulomatous tissue and rarely showed evident aspects of activation. Moreover, B-cell blast aggregates were much rarer than in the +/+ mice. It is likely that these differences are directly due to the absence of IL-6, which is normally abundantly produced in the peritoneal cavity of pristane-treated mice (reviewed in Ref. 19), probably mainly by macrophages/histiocytes and fibroblasts and possibly by lymphocytes. These data are in agreement with recent work by Anderson and collaborators, who have shown that, in IL-6-deficient mice of the C57 Black 6 genetic background, which is known to be resistant to plasmacytoma development, pristane oil injection triggers a reduced inflammatory response and no plasma cell hyperplasia.[25]

There was a critical period of approximately 8 weeks starting from day 162 after the first pristane oil injection during which the cellular composition of the ascitic fluids in the BIL-6+/+ mice dramatically changed, due to a sudden increase in the number of lymphocytes and histiocytes, and during this period the first foci of atypical plasma cells started to appear. Strikingly, these changes were completely absent in the BIL-6−/− mice. These sudden and dramatic changes are likely to be the result of two combined phenomena: 1) the accumulation of a sufficient number of antigenic stimuli, as it is known that exposure to antigens is required for the development of plasmacytomas (reviewed in Ref. 19) and 2) a positive feedback loop of IL-6 on its own production, determining an increasing number of histiocytes to become activated and to produce IL-6, which in turn exerts its stimulating effects on lymphocyte proliferation and differentiation. Although the antigenic stimuli occurred also in the IL-6−/− mice, and IL-6 was not required for lymphocyte proliferation and plasma cell differentiation as shown by the detection of both B-cell blasts aggregates and of mature plasma cells in the BIL-6−/− mice, clearly in the absence of IL-6 a fundamental stimulus triggering uncontrolled lymphocyte proliferation was missing.

The exact mechanisms leading to neoplastic transformation and the development of plasmacytomas in response to pristane oil treatment are not completely understood. However, they are thought to include a combination of antigenic stimuli promoting B cell differentiation and isotype switching and the influence of the peritoneal granuloma microenvironment, which favors both proliferation and prolonged survival of antigenically activated blast cells (reviewed in Ref. 19). Interestingly, IL-6 is not only a growth factor for myeloma and plasmacytoma cells, but it is also known to act as an anti-apoptotic factor.[26–28] It was recently shown that counteracting IL-6 action by means of receptor antagonists could induce not only growth arrest but also irreversible death by apoptosis in MM cells.[29] Therefore, in an envi-

ronment promoting continuous blast cell proliferation, IL-6 could also be responsible of inhibiting apoptosis of proliferating B-cell clones that would otherwise be destroyed, thus favoring survival, amplification, and maturation of both normal and neoplastic B cells.

IL-6-mediated prolongation of B-cell clone survival may play an important role in increasing the probabilities of neoplastic transformation and of tumor development. However, we have some indication that tumor transformation may occasionally occur also in the BIL-6−/− mice, as plasma cells with atypical or anaplastic features could occasionally be spotted both in the ascitic fluids and in the mesenteric tissues of some of these mice (Figure 5). The intense staining of these atypical plasma cells for kappa light chains with no reaction for lambda light chains and the striking similarity of the pattern obtained with that of plasmacytoma-affected BIL-6−/− mice supports the hypothesis of an initial plasma cell transformation. Although these observations are exclusively based on morphological data and therefore need to be confirmed at the molecular level by additional studies, they seem to suggest that the stochastic transformation event can occur independently of IL-6 but is not able to give origin to a developed tumor in the absence of this cytokine.

Our data also indicate that, although cytokines such as IL-10 and those belonging to the IL-6 family and signaling through the common receptor subunit gp130 are able to support the growth of certain MM and plasmacytoma cell lines and were therefore proposed to play a role in the pathogenesis of these diseases,[30,31] none of these cytokines can substitute for IL-6 function *in vivo*. These results confirm and expand previously published data showing that IL-6-deficient mice did not develop B-cell tumors when infected with a *myc/raf*-expressing retrovirus and could not support the *in vivo* growth of an established plasmacytoma cell line.[18] Our data highlight the importance of IL-6 in the development of B-cell neoplasias in mice also in the absence of directly transforming events and support the view that the development of efficient inhibitors of IL-6 activity may be a very important pharmacological tool for the cure of MM in human patients. Interestingly, the development of IL-6-independent plasmacytomas has been recently shown to be triggered by a *myc/abl* retrovirus, apparently through constitutive activation of the IL-6 signaling pathway in the tumor cells.[32] This finding, although further highlighting the importance of IL-6-mediated functions for the development of B-cell neoplasias, indicates a possible mechanism for the generation of IL-6-independent tumors also in humans and calls for additional efforts focusing on interference with the IL-6 signaling pathway.

Acknowledgments

We thank Dr. Patrizia Costa for her important input into the organization of the experiment and Sabrina Germoni and Simonetta Di Carlo for animal care.

References

1. Kishimoto T, Hirano T: Molecular regulation of B lymphocyte response. Annu Rev Immunol 1988, 6:485–512
2. Hirano T, Akira S, Taga T, Kishimoto T: Biological and clinical aspects of interleukin 6. Immunol Today 1990, 11:443–449
3. Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Costantini F: Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J 1994, 13:1189–1196
4. Klein B: Cytokine, cytokine receptors, transduction signals, and oncogenesis in human multiple myeloma. Semin Hematol 1995, 32:4–19
5. Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, Asaoku H, Tang B, Tanabe O, Tanaka H, Kuramoto A, Kishimoto T: Autocrine generation and requirement of BSF-2/IL6 for human multiple myelomas. Nature 1988, 332:83–85
6. Klein B, Zhang XG, Jourdan M, Content J, Houssiau F, Aarden L, Piechaczyk M, Bataille R: Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. Blood 1989, 73:517–526
7. Zhang X-G, Gaillard J-P, Robillard N, Lu Z-Y, Gu Z-J, Jourdan M, Boiron JM, Bataille R, Klein B: Reproducible obtaining of human myeloma cell lines as a model for tumour stem cell study in human multiple myeloma. Blood 1994, 83:3654–3663
8. Bataille R, Jourdan M, Zhang XG, Klein B: Serum levels of interleukin-6, a potent myeloma cell growth factor, as a reflect of disease severity in plasma cell dyscrasias. J Clin Invest 1989, 84:2008–2011
9. Nachbaur DM, Herold M, Maneschg A: Serum levels of interleukin-6 in multiple myeloma and other hematological disorders: correlation with disease activity and other prognostic parameters. Ann Hematol 1991, 62:54–58
10. Reibnegger G, Krainer M, Herold M, Ludwig H, Wachter H, Hubert H: Predictive value of interleukin-6 and neopterin in patients with multiple myeloma. Cancer Res 1991, 51:6250–6253
11. Klein B, Wijdenes J, Zhang X-G, Jourdan M, Boiron J-M, Brochier J, Liautard J, Merlin M, Clement C, Morel-Fournier B, Lu Z-Y, Mannoni P, Sany J, Bataille R: Murine anti-IL-6 monoclonal antibody therapy for a patient with plasma cell leukemia. Blood 1991, 78:1198–1204
12. Bataille R, Barlogie B, Lu ZY, Rossi J-F, Lavabre-Bertrand T, Beck T, Wijdenes J, Brochier J, Klein B: Biologic effects of anti-interleukin-6 murine monoclonal antibody in advanced multiple myeloma. Blood 1995, 86:685–691
13. Klein B, Zhang X-G, Lu Z-Y, Bataille R: Interleukin-6 in human multiple myeloma. Blood 1995, 85:863–872
14. Nordan RP, Potter M: A macrophage-derived factor required by plasmacytomas for survival and proliferation in vitro. Science 1986, 233:566–569
15. Van Snick J, Vink A, Cayphas S, Uyttenhove C: Interleukin-HP1, a T cell-derived hybridoma growth factor that supports the in vitro growth of murine plasmacytomas. J Exp Med 1987, 165:641–649
16. Degrassi A, Hilbert DM, Rudikoff SA, Anderson OM, Potter M, Coon HG: In vitro culture of primary plasmacytomas requires stromal cell feeder layers. Proc Natl Acad Sci USA 1993, 90:2060–2064
17. Suematsu S, Matsusaka T, Matsuda T, Akira S, Ohno S, Miyazaki J, Yamamura K, Hirano T, Kishimoto T: Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice. Proc Natl Acad Sci USA 1992, 89:232–235
18. Hilbert DM, Kopf M, Mock BA, Köler G, Rudikoff S: Interleukin 6 is essential for in vivo development of B lineage neoplasm. J Exp Med 1995, 182:243–248
19. Potter M, and Wiener F: Plasmacytomagenesis in mice: model of neoplastic development dependent upon chromosomal translocations. Carcinogenesis 1992, 13:1681–1697
20. Hilbert DM, Shen M-Y, Rapp UR, Rudikoff S: T cells induce terminal differentiation of transformed B cells to mature plasma cell tumors. Proc Natl Acad Sci USA 1995, 92:649–655
21. Potter M, Wax JS: Peritoneal plasmacytomagenesis in mice: comparison of different pristane dose regimens. J Natl Cancer Inst 1983, 71:391–395
22. Potter M: Genetics of susceptibility to plasmacytoma development in Balb/c mice. Cancer Surv 1984, 3:247–264
23. Fattori E, Cappelletti M, Costa P, Sellitto C, Cantoni L, Carelli M, Faggioni R, Fantuzzi G, Ghezzi P, Poli V: Defective inflammatory response in interleukin 6-deficient mice. J Exp Med 1994, 180:1243–1250

24. Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, Faggioni R, Luini W, Van Hinsberg V, Sozzani S, Bussolino F, Poli V, Ciliberto G, Mantovani A. Immunity 1997, 6:315–325
25. Dedera DA, Urashima M, Chauhan D, LeBrun DP, Bronson RT, Anderson AC: Interleukin-6 is required for pristane-induced plasma cell hyperplasia in mice. Br J Hematol 1996, 94:53–61
26. Hardin J, MacLeod S, Grigorieva I, Chang R, Barlogie B, Xiao H, Epstein J: Interleukin-6 prevents dexamethasone-induced myeloma cell death. Blood 1994, 84:3063–3070
27. Kawano MM, Mihara K, Huang N, Tsujimoto T, Kuramoto A: Differentiation of early plasma cells on bone marrow stromal cells requires interleukin-6 for escaping from apoptosis. Blood 1995, 85:487–494
28. Schwartze MMK, Hawley RG: Prevention of myeloma cell apoptosis by ectopic bcl-2 expression or interleukin 6-mediated up-regulation of bcl-x_L. Cancer Res 1995, 55:2262–2265
29. DeMartis A, Bernassola F, Savino R, Melino G, Ciliberto G: Interleukin 6 receptor antagonists are potent inducers of human multiple myeloma cell death. Cancer Res 1996, 56:4213–4218
30. Lu ZY, Zhang XG, Rodriguez C, Wijdenes J, Gu ZJ, Morel-Fournier B, Harousseau JL, Bataille R, Rossi JF, Klein B: Interleukin-10 is a proliferation factor but not a differentiation factor for human myeloma cells. Blood 1995, 85:2521–2527
31. Zhang XG, Gu ZJ, Lu ZY, Yasukawa K, Yancopoulos GD, Turner K, Shoyab M, Taga T, Kishimoto T, Bataille R, Klein B: Ciliary neurotropic factor, interleukin 11, leukemia inhibitory factor, and oncostatin M are growth factors for human myeloma cell lines using the interleukin 6 signal transducer gp130. J Exp Med 1994, 179:1337–1342
32. Hilbert DM, Migone T-S, Kopf M, Leonard WJ, Rudikoff S: Distinct tumorigenic potential of *abl* and *raf* in B cell neoplasia: *abl* activates the IL-6 signaling pathway. Immunity 1996, 5:81–89