Introduction

In all murine plasmacytomas (MPCs), expression of the \( c-myc \) gene is deregulated irrespective of the strain origin or induction system. In the majority of MPCs, dysregulation of the \( c-myc \) transcript is achieved by chromosomal translocation that juxtaposes the \( c-myc/Pvt-1 \) locus on chromosome 15 with one of the immunoglobulin (Ig) loci on chromosomes 12 (IgH), 6 (Igκ) or 16 (Igλ) (1–4). In a few translocation-free MPCs, molecular analysis revealed that the \( c-myc \) gene was constitutively activated by retroviral long terminal repeat insertion 5’ of the gene (5). MPCs that evolved in mice infected with retroviral constructs or in transgenic mice containing constitutively activated \( c-myc \) or \( v-myc \) genes are also translocation free (6,7). The consistent finding that \( c-myc \) expression is deregulated in all MPCs indicated that it is a critical event obligatorily required for transformation of B lymphocytes into MPC. However, neither the timing and the mechanism of the MPC-associated translocation nor the differentiation status of the precursor cells in terms of their developmental stage has been unambiguously defined. The most distinctive feature observed in the incidence of MPC-associated chromosomal translocation is that the ratio of the typical t(12;15) versus the variant t(6;15) translocation varies considerably with different induction methods (8). More than 90% of pristane-induced MPCs carry the t(12;15) translocation, whereas the percentage of the t(6;15) translocation was <10%. However, when MPCs were induced with pristane plus Abelson murine leukemia virus (A-MuLV), the incidence of MPCs carrying the t(6;15) translocation increased up to 40%. The shift in incidence was conspicuous when MPCs were induced by pristane plus A-MuLV in congenic BALB/cRb6.15 (B/cRb6.15 mice), which carry a pair of Robertsonian (Rb) translocation chromosomes. The ratio of the typical t(12;15) versus the variant t(6;15) translocation changed significantly to 30 and 70%, respectively (F.Wiener, unpublished data).

These findings raise an intriguing question as to whether the MPC precursor cells carrying either a typical or a variant type of translocation are in different stages of B cell differentiation. We hypothesized that the generation of t(12;15) translocation is restricted to precursor cells at a late stage of differentiation, to immature/mature B cells and also plasma cells, while the variant types, t(6;15) and t(15;16), occur in cells at a rather early stage of differentiation, such as B cells, while the variant types, t(6;15) and t(15;16), occur in cells at a rather early stage of differentiation, such as B cell progenitor (pre-B) and precursor B (pre-B) cells. This hypothesis is based on the assumption that the Ig and \( c-myc \) genes involved in such illegitimate interchromosomal rearrangements, being active, would be simultaneously transcribed, since their ‘open’ chromatin is accessible to the action of the recombinase enzymes (9,10). In B cells such a situation occurs during the differentiation process from the pre-B through the pre-B cell when the Ig light and heavy chain genes undergo rearrangements (11–13). Accordingly, translocation with the breakpoint in a switch sequence would occur in mature B cells (IgM/D, IgG, IgA) and/or plasma cells residing in the peripheral organs of the immune system such as spleen, lymph node (LN) and Peyer’s patches. On the other hand, the chance of Igκ/myc and Igλ/myc juxtaposition is probably much higher in pre-B cells.
higher in pre-B cells, where rearrangement of the IgL chain gene is under way. This would imply that translocations involving the VJ light chain and Pyy-l sequences would take place in an early pre-B cell primarily in the bone marrow (4).

In an attempt to associate the translocation event and the ability to undergo malignant transformation with a more defined population of precursor cells, we designed MPC induction experiments in C.B-17 scid/scid (SCID) mice reconstituted with B lineage cells of BALB/c mouse origin characterized by stage-specific differentiation markers. The SCID mouse is homozygous for a recessive autosomal mutation which is expressed as a severe immunological deficiency and almost complete absence of functional B and T cells (14). B cell differentiation is blocked at the late pro-B stage (15,16). The SCID mouse is virtually depleted of MPC precursor cells and consequently no MPCs will develop in this strain. It has been shown, however, that SCID mice can be reconstituted with functional lymphocytes by transplantation of fetal liver or bone marrow cells from normal donor BALB/c mice. Such mice are immunologically competent and when successfully reconstituted with BALB/c-derived hematopoietic cells, the SCID mice developed MPCs with a similar frequency to that of conventional BALB/c mice (F.Wiener, unpublished data).

In this study, we report the results of MPC induction experiments in SCID mice reconstituted with either surface Ig-positive (sIg+) B lineage cells obtained from spleens of conventional B/cRb6.15 or with sIg-negative (sIg-) plasma cells from the LNs of human IL-6 transgene-congenic BALB/cRb8.12 (B/cRb8.12 IL-6-Tg) mice. The presence of specific chromosomal markers, Rb6.15 or Rb8.12, in the cell population used for reconstitution allowed us to accurately distinguish the donor origin of the MPCs. Altogether, the data obtained strongly suggest that the malignant transformation initiated by deregulation of the myc gene occurs preferentially in sIg+ B cells rather than in differentiated, G0 or cycling plasma cells. We thus inferred that immature and/or mature B cells and not differentiated plasma cells are most likely the principal source of precursor cells from which typical t(12;15) MPCs develop.

Materials and methods

Mice

Female SCID mice were purchased from the Central Institute for Experimental Animals (Kawasaki, Japan) and were housed in laminar flow animal isolation hoods. They were fed with sterile food and water. B/cRb6.15 mice were obtained from the Microbiology and Tumorbiology Center, Karolinska Institute (Stockholm, Sweden) and were housed in the specific pathogen-free animal facility. The B/cRb6.15 mouse is a congenic strain of BALB/c characterized by the presence of two copies of Rb chromosome 6.15 (Rb6.15) of AKR origin (17). The BALB/cRb8.12 (B/cRb8.12) mouse, provided by F.Wiener (MTC, Karolinska Institute, Stockholm, Sweden), is a congenic strain of BALB/c with two copies of centromerically fused Rb chromosome 8.12 chromosomes of Rb8.12/Rb origin (19). Both strains develop MPCs with an incidence of 30–60%, similar to that of conventional BALB/c mice. B/cRb8.12 IL-6-Tg mice were produced in our animal facility by repeated backcrossing of a human IL-6 transgenic mouse of C57BL/6 (B6) origin (20) into a MPC-prone B/cRb8.12 background. The transgene carrier mice produce predominantly IgG1 protein. Mice used in all of the experiments complied with the standards set out in the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi campus of Kanazawa University.

Cell separation

sIg+ B lineage cells were prepared from B/cRb6.15 mouse spleens at 8–10 weeks of age by a slightly modified panning method (21). Briefly, a cell suspension of 10–12 spleens in phosphate-buffered saline (PBS) containing 5% fetal calf serum (FCS) was centrifuged and the cell pellet was treated for 5 min at 0°C in ACK solution (8.28 g NH4Cl, 1 g KHCO3, 0.04 g EDTA in 1000 ml distilled water, pH 7.4). After centrifugation, the cell pellet was resuspended in PBS with 5% FCS and was incubated at 37°C for 1 h in uncoated plastic plates (10 cm diameter, 2×105 cells/5 ml/dish; Nunc, Roskilde, Denmark) to remove the cells prone to adhere spontaneously to the plastic dish. To separate the sIg+ B cells the cell suspension was layered onto Petri dishes (1×105 cells/5 ml/dish) precoated with rabbit anti-mouse IgS which reacts with all classes of mouse Ig. The dishes had been precoated overnight at 4°C with 5 ml antibody solution containing 25 µg/ml (Dako, Glostrup, Denmark) in PBS with 5% FCS. The cells were allowed to settle at room temperature for 1 h. After removing the non-adherent cells, the dishes were gently washed twice with PBS and the adherent cells on the bottom surface were collected in PBS with 5% FCS. The second cycle of incubation was performed in the same manner on additional antibody-coated plates. The plasma cell-rich fraction was obtained from the LNs of N6 backcross generation B/cRb8.12 IL-6-Tg mice, 15–20 weeks of age. After removing adherent cells, the cell suspension was treated according to the protocol applied for the selection of sIg+ B cells and the non-adherent sIg- cells were used as a plasma cell-rich population.

Reconstitution of SCID mice

Reconstitution of 8–10-week-old female SCID mice was carried out by i.v. injection of 1×108 sIg+ cells/mouse together with the same number of thymocytes obtained from 1-month-old B/cRb6.15 mice or by i.v. injection of 1×106 plasma cell-rich population/mouse. No T lymphocytes were added to this fraction because the plasma cell-rich population contained ~20% T lymphocytes (see Results).

MPC induction

MPCs were induced in the reconstituted SCID mice according to one of the following schedules. (i) One week after reconstitution, the mice were inoculated i.p. with 0.5 ml pristane followed 2 weeks later by an i.p. injection of 1×106 focus-forming units (0.25 ml) of A-MuLV (92) (provided by Dr E.B.Musinsky, NCI, Laboratory of Genetics, Bethesda, MD). Additional 0.5 ml pristane treatments were repeated twice every 2 months. (ii) One week after reconstitution, the mice were inoculated i.p. with 0.5 ml pristane three times at 2-monthly intervals. MPCs were induced in two control groups comprising 20 SCID mice reconstituted with sIg+ B cells obtained from B/cRb8.12 IL-6-Tg mouse spleens and 10 conventional (non-reconstituted) B/cRb8.12 IL-6-Tg mice, respectively, by i.p. injection of 0.5 ml pristane three times at 2-monthly intervals.

Antiserum

To characterize the B lineage cells prepared by panning, cell surface antigens were stained with FITC-conjugated goat anti-mouse IgM (Vector, Burlingame, CA), rat anti-mouse Thy-1.2 (PharMingen, San Diego, CA) and PE-coupled rat anti-mouse Thy-1.2 (PharMingen, San Diego, CA) and PE-coupled rat anti-mouse CD43 (S7) (PharMingen) was followed by avidin–FITC. Staining with biotinylated rat anti-mouse CD43 (S7) (PharMingen) was followed by avidin–FITC. To detect all classes of mouse Ig, purified cells were first incubated with polyclonal rabbit anti-mouse Ig (Dako) reacting with all the mouse Ig isotypes and then stained with biotinylated goat anti-rabbit IgG and avidin–FITC. Cytoplasmic IgG1 was stained with biotinylated rat anti-mouse IgG1 (Immunotech, Marseille, France) following avidin–FITC. Circulating IgG1 levels of the plasma cell-reconstituted SCID mice were determined by ELISA (22) using horseradish peroxidase-conjugated sheep anti-mouse IgG1 (Binding Site, Birmingham, UK) as a second antibody. Allotyping of the serum IgG1 produced in these mice was performed by immunoelectrophoresis of papain-digested sera (23).

Chromosome analysis

Metaphase spreads were prepared from spleen and LN cells of B/cRb8.12 IL-6-Tg mice (N6) and from cells of ascitic tumors which developed in the reconstituted SCID mice. G-banding analysis was performed as described previously (1). Chromosomes were identified according to the International Committee on Standardized Genetic Nomenclature for Mice (24).

Southern blot analysis

High molecular weight DNAs prepared from a B/cRb8.12 IL-6-Tg mouse spleens and 10 conventional (non-reconstituted) B/cRb8.12 IL-6-Tg mice were digested with EcoRI, BamHI or HindIII. Genomic Southern blot analysis was carried out using 32P-labeled S107 as a c-myc probe (25).

Simple sequence repeat length polymorphism (SSLP) analysis

SSLP loci were determined according to Nikaido et al. (26).

Polymerase chain reaction

The fate of the transplanted B/cRb8.12 IL-6-Tg mouse LN-derived plasma cells was followed by identifying the IL-6 transgene in the spleen cell population of the reconstituted SCID mouse. PCR was carried out using the following pair of primers: 5'-ACCTCTTCAGAACGAATTGACA AA-3' and 5'-AGCTGC-
Plasmacytomas with a t(12;15) translocation

Fig. 1. (A) Photomicrograph of a histopathology section of an LN from a B/cRb8.12 IL-6-Tg mouse. Note the monomorphic, massive plasmacytosis that replaced the normal structure of the LN. Hematoxylin and eosin (HE) staining. (B) Magnified region of the same section. Plasma cells with dense amphiphilic cytoplasm and eccentric nuclei with clockfaced chromatin and prominent nucleoli. (C and D) Cytospins of IgG1-producing plasma cell clusters stained with biotinylated rat anti-mouse IgG1 followed by avidin-FITC. Scale bar 20 µm.

GCAGAATGAGATGAGTTGT-3'. Amplification consisted of 32 cycles of 1 min denaturation at 95°C, 1 min annealing at 55°C and 30 s extension at 75°C.

Results

Pathological changes, chromosomal analysis, SSLP and allotyping of the B/cRb8.12 IL-6-Tg mice

The B/cRb8.12 IL-6-Tg mice used as donors for the plasma cell population were at N6 backcross, consistent with a BALB/c background of 98%. The pathological changes in the original human IL-6 transgenic B6 mice have been described elsewhere (27). Essentially the same pathological changes were observed in the B/cRb8.12 IL-6-Tg mice. All mice displayed splenomegaly and peripheral adenopathy, in particular enlargement of the axillary and inguinal LNs. The LNs were almost entirely replaced by plasma cells characterized by an eccentric nucleus with prominent speckles of chromatin and cytoplasm with a marked peripheral basophilia (Figure 1A and B).

To determine the degree of genetic identity between the B/cRb8.12 IL-6-Tg and B/cRb8.12 mice, we analyzed the chromosomal constitution of the transgenic mice and they were typed for a number of genomic markers and Ig allotype. The cytogenetic analysis confirmed that the chromosome constitution of the B/cRb8.12 IL-6-Tg mouse was identical to that of B/cRb8.12, i.e. it was homozygous for the Rb8.12 marker chromosome (Figure 2).

SSLP locus analysis revealed that the D1Mit14, D2Mit30, D3Mit14, D4Mit15, D6Mit1, D6Mit15, D7Nds1, D8Mit4, D10Mit11, D11Mit5, D11Mit20, D11Mit21, D12Mit5, D12Mit7, D12Nds12, D13Mit3, D13Mit13, D14Mit7, D15Mit28, D15Mit46, D16Mit4, D17Mit21, D19Mit4 and DXMit1 sequences were identical to those of the B/cRb8.12 mouse. The IgG1 detected in the sera of the transgene-congenic mice was of ‘a’ allotype, as was that of the B/cRb8.12 mouse.

Fig. 2. G-banded karyotype of an LN cell of a B/cRb8.12 IL-6-Tg mouse. Note the presence of the B/cRb8.12 mouse-derived Rb8.12 homolog chromosome pair and the diploid chromosome constitution.
In addition to the sIgF with the phenotype of immature and mature B cells (11,12). These characteristics of surface antigen expression are consistent with the phenotype of immature and mature B cells (11,12). Similarly, the major histocompatibility H-2\(^b\) complex of the original IL-6 transgenic B6 mouse was successfully replaced at the N6 backcross with the H-2\(^d\) complex of BALB/c origin (data not shown).

**Characterization of sIg \(^+\) B lineage and plasma cells used for reconstitution of the SCID mice**

For separation of sIg\(^+\) cells we applied the panning technique (21) because flow cytometry did not provide a sufficient number of cells for successful reconstitution of a total of 140 SCID mice (groups 1–3). Immunofluorescence analysis of the antigens expressed on the surface of the cells separated by panning is summarized in Table I. The sIg\(^+\) cells were >90\% IgM\(^+\), 99\% B220\(^+\), <1\% BP-1 and CD43 dull\(^+\) and Thy-1\(^-\). These characteristics of surface antigen expression are consistent with the phenotype of immature and mature B cells (11,12). In addition to the sIg\(^+\) cells, a small number of cells were sIgG\(^+\) or sIgA\(^+\), since we used a polyclonal anti-mouse Ig for panning of the spleen cell population (data not shown).

The plasma cell-rich population was obtained from the LNs of B/cRb8.12 IL-6-Tg mice after removing the sIg\(^+\) cells by panning as described in Materials and methods. The non-adherent cell fraction contained mainly large cells (~70\%) and was characterized by Thy-1\(^-\), B220\(^-\), sIgM\(^-\) and BP-1\(^-\) cells. Immunofluorescence staining of the cell fraction indicated that nearly all of the large cells were IgG1-producer plasma cells. Thus, both the morphological staining and immunotyping corroborated that the non-adherent cell fraction consisted of T lymphocytes and IgG1-producer plasma cells (Figure 1C and D).

**DNA blot analysis of LN cells of IL-6 transgenic BALB/cRb8.12 mice**

To exclude the occurrence of spontaneous genomic alterations, primarily c-myc rearrangements, in the cell population manifesting massive plasmacytosis of IgG1-producer cells (Figure 1), we performed Southern blots of EcoRI, BamHI and HindIII digests of genomic DNA obtained from the LNs of a B/cRb8.12 IL-6-Tg mouse. Hybridization with the c-myc probe (S107) revealed that none of the digests displayed rearrangement in the c-myc locus (Figure 3).

**Plasmacytoma induction and development in the reconstituted SCID mice**

The results of the induction experiments are summarized in Tables II–IV. MPCs were induced in the SCID mice reconstituted with sIg\(^+\) cells by pristane plus A-MuLV (group 1) and by pristane alone (group 2). As shown in Table II, no significant differences in incidence or latency for MPC development were detected between the two experimental groups, irrespective of the induction method. The mean latency in both groups was 180 days (data not shown), indicating that the accelerated MPC genesis characteristic of the pristane plus A-MuLV induction (28) was not manifest in any of the experimental groups. Similarly, the chromosomal analysis of MPCs demonstrated that all were of the typical (12;15) translocation type (Figure 4). None of the MPCs carried the variant (t6;15) translocation which is frequently encountered in tumors induced by pristane plus A-MuLV. The presence of the Rb6.15 marker chromosome in the MPCs confirmed unequivocally their donor origin.

Induction experiments performed in a group of 20 SCID mice reconstituted with sIg\(^+\) cells originating from B/cRb8.12 IL-6-Tg mice (Table III, group 3) aimed to test whether the cells containing the IL-6 transgene could serve as MPC precursors. The development of donor type MPCs in the reconstituted SCID mice (Figure 5) demonstrated that integration and expression of the IL-6 transgene in the genome of the sIg\(^+\) cells does not interfere with MPC genesis. These cells possess similar potential to develop into MPCs as those derived from conventional B/cRb8.12 mice. These results are also substantiated by the MPC induction experiments performed in B/cRb8.12 IL-6-Tg mice (Table III, group 4). As expected, pristane treatment of the transgene-congenic mice triggered MPC development similar to that observed in reconstituted SCID mice of group 3. The MPCs developing in both groups 3 and 4 were also carriers of the typical (t12;15) translocation.

The results of the induction experiments for group 5 (Table IV) are of paramount importance. The SCID mice were reconstituted with the plasma cell-rich population of the B/cRb8.12 IL-6-Tg mouse LNs. Irrespective of the induction method, neither MPCs nor any other type of tumor developed in 48 SCID mice during the 1 year observation period.

Fisher’s exact tests based on direct calculation of the probabilities in a 2×2 table were applied to test the significance of differences in MPC incidence in groups 1–3 (reconstituted with sIg\(^+\) B cells), group 4 (in situ induction) and group 5 (plasma cell-reconstituted). The following probabilities were

| Table I. Characterization of the cells either adherent or non-adherent to the anti-Ig-coated plates |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                   | BP-1 | CD43 | B220 | sIgs | IgM | Thy-1.2 |
| Adherent cells    | <1   | <1   | 99%  | 99%  | 90% | <1    |
| Non-adherent cells| 50   | <5   | <1   | <1   | -20 | <1    |

Data represent the % positive cells.
obtained; group 5:group 1, 0.0829 (P < 0.1); group 5:group 2, 0.0392 (P < 0.05); group 5:group 3, 0.0834 (P < 0.1); group 5:group 4, 0.0272 (P < 0.05). Thus, the hypothesis that MPC incidences in these experimental groups are identical is evidently disproved, although the number of MPCs in each group is relatively small.

**Persistence of plasma cells transferred into SCID mice**

Survival of the transferred plasma cells was monitored by: (i) measurement of circulating IgG1 levels in the sera of the reconstituted SCID mice; (ii) histopathological studies; and (iii) PCR analysis of spleen tissues. Amounts of serum IgG1 measured by ELISA in a group of eight plasma cell-reconstituted SCID mice showed a substantial increase until 4 months after reconstitution, then started to decrease. Even 6 months after reconstitution it was several log higher than the amount of serum IgG1 in the non-reconstituted control SCID mice. Mouse 5-1 showed the lowest amount of serum IgG1. Even in this mouse, the values were 11.8 mg/ml on day 121 and 34.9 mg/ml on day 183 post-cell transfer. These amounts of serum IgG1 are comparable with those found in IgG1 myelomas of mice (Figure 6). Immuno-electrophoretic analysis of the papain-digested sera (23) of these reconstituted mice showed IgG1 of ‘a’ allotype (data not shown). This clearly indicates a B/cRb8.12 IL-6-Tg origin, since it is distinct from the ‘b’ allotype of SCID mice.

The reconstituted SCID mice displayed a massive splenomegaly but curiously enough no peripheral lymphadenopathies were observed. Histopathological studies revealed the presence of large clusters of IgG1-secreting plasma cells scattered throughout the pulp tissue of the enlarged spleen (Figure 7C and D), in contrast to the spleen of the control SCID mouse, which was completely depleted of plasma cells (Figure 7A and B). Presence of the human IL-6 transgene in the spleen cell population, revealed by PCR analysis, also confirmed the donor origin of the plasma cells (Figure 8). Altogether, the results substantiate large numbers of the transferred plasma cells surviving for months in recipient SCID mice, conceivably a period long enough for MPC development. Moreover, cytogenetic analysis revealed that cells carrying the Rb8.12 marker chromosome are still dividing even after 6 months in the enlarged spleen of the reconstituted SCID mice (data not shown).
Discussion

Origin of MPC precursor cell

MPCs are tumors of terminally differentiated B lineage plasma cells induced by a variety of treatments in BALB/c and NZB inbred strains of mice (4). Constitutive activation of c-myc by chromosomal translocation is found in virtually all MPCs and it is presumed to occur randomly in B cells during physiological rearrangement of the Ig genes (8–13). Although the Ig/myc translocation is an indispensable step in MPC genesis, additional genetic mutations have to occur in the MPC precursor cell in order to fully acquire a malignant phenotype (4). The present study focuses on the B cell source of MPC precursor cells carrying the typical t(12;15) translocation. The morphological and physiological similarities between plasma cells and the differentiated and rapidly growing plasmacytoma cells support the concept that the plasma cell is likely to be the precursor cell from which the typical t(12;15) MPCs develop. Our aim was to determine whether the ability to undergo malignant transformation and subsequently to serve as a MPC precursor is restricted to differentiated plasma cells or whether immature or mature Ig-positive B cells in the process of switching are the legitimate precursors from which MPCs develop. Our previous studies (19) have uncovered the presence of an IgSα/myc juxtaposition in preneoplastic OG tissues in as many as 73% of BALB/c mice as early as 30 days following pristane treatment. Although the PCR methodology was unable to define the differentiation stage of the cells carrying the IgSα/myc recombination, it was hard to escape the conclusion that the focal plasma cell proliferation contains the precursor cells from which MPCs develop.

Survival of IL-6-Tg plasma cells in the reconstituted SCID mice

In the present study, by employing the cell transfer approach, we designed a number of experiments to decide whether slg+ B or differentiated plasma cells serve as the MPC precursor cell, which subsequently undergoes malignant transformation. The prerequisite of the transfer experiments was assessing whether the resettled B cell or plasma cell population survived in the new environment, i.e. in the pristane-treated SCID host.

Since MPCs developed solely from transferred slg+ B cells and not from the IL-6-Tg plasma cells, we only investigated the fate of plasma cells in the reconstituted SCID hosts. The eventual lack of MPC development might well be attributed...
MPCs revealed that 40% were of variant t(6;15) and t(15;16) type, while in 81 induced by pristane alone only 10% were carriers of the variant translocation (F.Wiener, unpublished data). The much higher incidence of variant translocation in the pristane plus A-MuLV MPCs versus those induced with pristane alone may be due to the inability of A-MuLV to efficiently infect immature and/or mature B cells (33).

A-MuLV predominantly infects pre-B cells and, like a mitotic catalyst, prompts a massive proliferation of the pre-B cell population in which the IgL chain gene rearrangements occur. Thus, the chance of mitotic errors resulting in accidental IgL/myc recombination increases significantly. This process may account for the high number of variant t(6;15) and t(15;16) MPCs in the pristane plus A-MuLV induction system. Conversely, since in all of the sIg^+ cells used for reconstitution experiments (groups 1–3) the light chains had already been rearranged, the likelihood of reiterating the illegitimate recombination between IgL and c-myc sequences is probably negligible. The accelerated MPC genesis characteristic of pristane plus A-MuLV induction (28) was not manifest in any of the experimental groups. Treatment of the reconstituted SCID mice with pristane alone or pristane plus A-MuLV resulted in the same mean latency of 180 days. The lack of an acceleration effect is also likely due to the inability of A-MuLV to infect and/or to integrate into the genome of sIg^+ cells. Consequently, the synergistic effect of the two oncogenes (c-myc and v-abl) evident in the pristane plus A-MuLV MPC genesis was also prevented. It is noteworthy that no pre-B lymphomas arose in experimental groups 1 (Table II) and 5 (Table IV) in spite of infection of the pristane-treated mice by A-MuLV. The use of a helper-free preparation of A-MuLV (ψ2) and the absence of pre-B cells in the reconstituted mice may account for the scarcity of lymphomas in these experimental groups.

**Ig/myc recombination does not interfere with the differentiation program of sIg^+ B cells**

An unusual aspect of MPC genesis in SCID mice reconstituted with sIg^+ cells was the emergence of a relatively high number of IgM-secretor MPCs. A similar pattern was found in Eµ-myc and N-myc transgenic mice and in mice infected with a retroviral construct comprising constitutively activated c-myc or v-myc (34,35). However, all MPCs were translocation free, i.e. the high levels of myc expression replaced the requirement for chromosomal translocation. MPCs induced in conventional experimental systems secrete predominantly IgA or IgG proteins. Only a few IgM- and IgD-secreting MPCs (<1%) that carry the t(12;15) translocation have been reported (36). In the present study, 40% of IgM-secreting MPCs developed in the sIg^+ B cell-reconstituted groups. Four out of 10 MPCs (40%) were IgM-secretors (Table II). As shown in Table I, >90% of sIg^+ cells used for reconstitution were sIgM^+.

Conceivably, Ig/myc recombination is an early event in this transfer system and that may explain the higher incidence of IgM-secretor MPCs. One may assume that Ig/myc recombination occurred in the sIgM^+ cells resulting mostly in MPCs secreting IgM and, in a few cases, IgG- or IgA-secretary MPCs. This aspect of MPC genesis implies that the translocation does not interfere with the differentiation program of immature/mature B cells, i.e. does not prevent sIg^+ B cells from evolving into highly differentiated Ig protein-secretor MPCs.

**IL-6-Tg plasma cells cannot serve as MPC precursors**

Inability of IL-6-Tg plasma cells to undergo malignant transformation was unambiguously proved by the results of induc-

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**Fig. 6.** The amount of serum IgG1 protein of allotype ‘a’ in the sera of eight SCID mice reconstituted with plasma cell-rich fraction obtained from the B/cRb8.12 IL-6-Tg mouse LNs. IL-6-Tg mouse-derived plasma cells are still secreting IgG1 6 months after transfer. Amount of serum IgG1 of the donor B/cRb8.12 IL-6-Tg mouse was 53.4 mg/ml at the time of reconstitution of the SCID mice. 5-0 and 6-0, non-reconstituted control SCID mice.

**Transfer of sIg^+ B cells results in the development of MPCs solely carrying the typical t(12;15)**

An interesting outcome of this study is that all MPCs, without exception, were carriers of the t(12;15) translocation, irrespective of whether the MPCs were induced by pristane plus A-MuLV or pristane alone. A compilation of previous cytogenetic analyses of 85 pristane plus A-MuLV-induced
Fig. 7. (A and B) Photomicrographs of a histopathological section of the spleen of an 8-month-old conventional SCID mouse. Note the spleen entirely depleted of plasma cells. (C and D) Histopathological section of the spleen of an 8-month-old SCID mouse reconstituted with plasma cells of B/cRb8.12 IL-6 Tg origin. Note the massive plasmacytosis in the pulp tissue of the spleen. Scale bar 20 μm.

Fig. 8. Detection of the IL-6 transgene by the PCR approach in splenic cells of SCID mice 6 months after reconstitution with IL-6-Tg plasma cells. Lanes 1–4 and 6–7, reconstituted SCID mice; lanes 8 and 9, conventional (non-reconstituted) SCID mice; lanes 10 and 11, IL-6-Tg-positive and IL-6-Tg-negative controls, respectively. Lane 5 shows the size markers given in bp.

plasmacytosis experiments in SCID mice of experimental group 5 (Table IV). All mice remained tumor free during the observation period of 1 year. This is even more intriguing in view of the significant splenomegaly, which strongly suggests the occurrence of massive cell proliferation in the reconstituted SCID mice. In addition, the presence of mitotic plates indicates that the transferred IL-6-Tg-derived plasma cells have left the resting G0 stage but cannot undergo malignant transformation in the MPC-promoting environment of pristane-treated SCID mice. Moreover, the production of IL-6, which is of paramount importance for MPC development (37) is even increased due to overexpression of the IL-6 transgene.

Conversely, the same environment effectively stimulated proliferation of sIg+ B cells, which readily developed into MPCs irrespective of the BALB/c mouse origin (conventional or transgenic; Tables II and III, groups 1–3). Similarly, in situ development of MPCs in the B/cRb8.12 IL-6-Tg mice (Table III, group 4) also excluded the likelihood of any negative regulatory effect of the IL-6 transgene on MPC genesis. This unique feature of the IL-6-Tg plasma cell to resist malignant transformation is poorly understood at the present time.

In a recent study, Kovalchuk et al. (38) using a PCR assay have shown that IgH/c-myc exchange can be detected abundantly in B lymphocytes residing in LNs and Peyer’s patches of untreated BALB/cAnPt.Tg-hu-IL-6 mice. This finding suggests that in this respect IL-6-Tg plasma cells are similar to immature/mature IL-6-Tg B cells when transferred into the MPC-promoting environment of pristane-pretreated SCID mice. However, the question why plasma cells harboring the Ig/myc exchange do not develop into MPCs still remains.

The answer to this question is hypothetical. We surmise that since the environment into which the IL-6-Tg sIg+ B and IL-6-Tg plasma cells were transferred is identical, the difference must reside at the cell level. The regular occurrence of benign plasmacytosis in the peripheral lymphoid organs of both B/cRb8.12 IL-6-Tg and reconstituted SCID mice strongly suggests that plasma cells regain their cycling ability by constitutive activation of the c-myc gene. Although the latter may be sufficient to sustain continuous cell division, it is likely far from enough to trigger further malignant transformation of plasma cells. This would imply that commitment of the precursor cell to MPC genesis is more likely to occur in the early stages of B cell development rather than in a differentiated
B lineage plasma cell. This would coincide, as was suggested (4,39), with Ig recombination of light or heavy chain loci or immediately after Ig/myc recombination was completed. Alternatively, the failure of the IL-6-Tg plasma cell to develop into MPC can be attributed to genetic or epigenetic factors acting downstream of the first oncogenic mutation, thus suppressing the ability of the plasma cells to acquire additional genetic changes required for achievement of the malignant phenotype.

Although >90% of the cells used in the transfer experiments were sIgM+ 1%, some of the cells were sIg- (Table 1). Thus, each reconstituted SCID mouse repopulated with 1×107 sIg- cells also received 2×103 sIg- cells. The ability of the (t(12;15) translocation might also be generated in a sIg- cell population could not be excluded with certainty. It remains to be verified whether SCID mice reconstituted with sIg- pro-B and pre-B cells will develop typical t(12;15) MPCs or rather MPCs predominantly of the variant t(6;15) and t(15;16) types.

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