Case Reports

Diagnosis of transfusion-associated graft-versus-host disease by genetic fingerprinting and polymerase chain reaction

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A patient with Hodgkin's disease (clinical stage IIIB) received chemotherapy and total nodal irradiation. After the transfusion of filtered packed red cells, this patient developed transfusion-associated graft-versus-host disease (TA-GVHD). The genetic fingerprint of the patient's peripheral blood lymphocytes (PBLs) differed completely from that of her other body tissues. Normally, after transfusion, only the patient's own genetic fingerprints are observed in the PBLs, as exemplified in more than 10 control cases in which the transfused blood had not been filtered before transfusion. No signal bands corresponding to those of the blood donor could be demonstrated in samples of the patient's tissue DNA. Moreover, chimerism was detected in the hybridization pattern of the patient's PBLs on the ninth day after the onset of symptoms. Polymorphic simple repeats in the HLA-DRB gene after amplification by polymerase chain reaction were also investigated, which confirmed the fingerprinting results. The advantages of these methods for the diagnosis of TA-GVHD include the rapid and unequivocal diagnosis as well as the fact that there is no need for the relatives to be HLA typed. TRANSFUSION 1992;32:766–770.

Abbreviations: bp = base pair; PBL(s) = peripheral blood lymphocyte(s); PCR = polymerase chain reaction; TA-GVHD = transfusion-associated graft-versus-host disease.

TRANSFUSION-ASSOCIATED graft-versus-host disease (TA-GVHD) can result when immunocompetent lymphocytes are transfused to immunoincompetent patients. At particular risk are neonatal patients, children with immune deficiency syndrome, and patients with hematologic malignancy or who have had chemotherapy, irradiation, or open heart surgery. However, even patients not normally regarded as immunocompromised can suffer from TA-GVHD (resulting, for example, from transfusion of blood from first-degree relatives to persons who are homozygous at class I loci).6

The typical clinical symptoms are associated with dysfunction of the liver and the gastrointestinal tract, such as diarrhea, skin rash, and fever up to 41° C. In addition, severe pancytopenia with bone marrow hypoplasia or aplasia has been reported. A diagnosis of TA-GVHD is established by HLA typing of peripheral blood lymphocytes (PBLs) and fibroblasts or of lymphocytes of both the transfusion recipient and the blood donor. Histologic examination of a skin biopsy confirms the diagnosis.

In this article, we present an alternative method for establishing the diagnosis of TA-GVHD: multilocus DNA fingerprinting. With this method, hypervariable DNA-fragment patterns that are distributed throughout the eukaryotic genome are investigated. In principle, several different multilocus probes could be used, such as minisatellites or synthetic oligonucleotide probes consisting of simple nucleotide repeats, as both provide an individual-specific banding pattern. However, the oligonucleotide probe (CAC)3 and its complementary equivalent (GTG)3 are most informative for man and are uncomplicated to handle.

An equivocal diagnosis can be obtained even faster by using a polymerase chain reaction (PCR)-based investigation of hypervariable regions in the genome that show stretches of simple repetitive sequences. In this case, we used the analysis of hypervariable intrinsic simple repeats in the HLA-DRB genes.

Case Report

A 23-year-old woman first presented with left cervical lymph node enlargement in March 1989. In July of the same year, Hodgkin's disease of the lymphocyte-rich type was diagnosed by biopsy. Enlarged lymph nodes were found on both sides of the diaphragm (clinical stage IIIB). Combined chemotherapy was therefore initiated with three cycles of cyclophosphamide,
Aspergillus fumigatus, vincristine, procarbazine, and prednisone and of adriamycin, bleomycin, dacarbazine, and vinblastine until January 1990. At that time, a lymph node relapse was observed and a new diagnosis made of Hodgkin’s disease of the nodular-sclerosing type. Since March 1990, the patient received total nodal irradiation (40 Gy). This cycle was interrupted several times, because of myelosuppression and subsequent transfusions of nonirradiated but filtered packed red cell transfusions (Sepacell R 500 A, Asahi, Tokyo, Japan). The last packed red cell transfusion was administered on August 1; 7 days later, the patient was hospitalized with an erythematous rash, fever to 41°C, and diarrhea, but no evidence of Hodgkin's relapse. All blood cultures remained negative. Yet Candida albicans and Aspergillus fumigatus were identified in the lung. Severe pancytopenia was present until the patient’s death. Her condition deteriorated in spite of several therapeutic regimens: cyclosporine A, corticosteroids, granulocyte-macrophage colony-stimulating factor to stimulate the stem cells, and T-cell receptor antibody (BMA 031, Behring, Marburg, Germany) in addition to antibiotics, antimycotics, and antiviral therapy. On September 4, the patient died of general heart and lung failure as a consequence of the fungal sepsis. The diagnosis of TA-GVHD was established on the basis of HLA typing of PBLs: mother, A2/3, B7/13, Cw6/7; father, A1/24, Bw4/w57, Cw3/-; and patient, A2/32, B7/w44, Cw4/7 (which is identical to the donor of 1 unit of the transfused packed red cells). At autopsy, residual Hodgkin’s disease was found in one cervical lymph node.

Materials and Methods

We performed the isolation of DNA from tissue and blood samples with normal WBC counts essentially according to the method of Sambrook and coworkers and the isolation of DNA from hair root cells according to the method of Teifel-Greding et al. Samples of blood from the patient (WBC counts approx. 400/μL) were incubated at 0°C with RBC-lysis buffer (2.7 mM [2.7 mmol/L] KCl, 137 mM [137 mmol/L] NaCl, 1.5 mM [1.5 mmol/L] KH₂PO₄, 7.75 mM [7.75 mmol/L] Na₂HPO₄, pH 7.4). Then we suspended the cells in 10 mM (10 mmol/L) Tris-HCl (pH 8.0), 10 mM (10 mmol/L) EDTA, and 150 mM (150 mmol/L) NaCl, added proteinase K and sodium dodecyl sulfate to a final concentration of 300 μg per mL and 0.6 percent, and incubated the mixture at 37°C. Standard phenol extraction and ethanol precipitation were performed. Afterwards we pelleted the DNA at 20,000 rpm for 45 minutes at 4°C (Beckman SW41 rotor) and dissolved the DNA in 10 mM (10 mmol/L) Tris-HCl, pH 8.0, and 1 mM (1 mmol/L) EDTA.

Up to 10 μg of each DNA sample was digested overnight by using HinfI (5 U/μg DNA) as recommended by the manufacturer (BRL, Egenstein, Germany). We carried out agarose gel electrophoresis (1 V/cm, 40 hours), labeling of the oligonucleotide probes (GTG)ₙ (Fresenius AG, Oberursel, Germany) with ³²P-ATP (Amersham, Braunschweig, Germany), and hybridization in the gel as described by Schäfer et al.

We amplified 1 μg of human genomic DNA by PCR for 30 cycles. The following HLA-DR oligonucleotide primer combinations were used: 1) O-DRw11.5/O-DRBrep annealing at 54°C and 2) O-DRw11.10/O-DRBre annealing at 50°C. They flank exon 2 of the DRB gene and part of intron 2 containing a single (g)ₙ, (gₙ), repeat sequence. The amplified DNA was separated on a 1-percent agarose gel to elute the 420-base pair (bp) (11.10/rep) and 448-bp (11.5/rep) fragment length ranges. We then labeled the isolated DNA with T₄ polynucleotide kinase and ³²P-ATP, separated the labeled DNA fragments on a 5-percent denaturing polyacrylamide gel, and autoradiographed or transferred them onto a nylon membrane (Hybond N, Amersham) by electroblotting. Afterwards the filter was hybridized with the internal HDB oligonucleotide at 59°C, washed throughly, and autoradiographed.

Results

To verify the suspected diagnosis of TA-GVHD, we compared the genetic fingerprint pattern of the PBLs with that obtained from the patient’s hair roots. The latter sample exhibited the patient’s constitutive banding pattern, while the pattern obtained from the PBLs was entirely different (Fig. 1A). Consequently, the circulating PBLs are derived from the donor of the transfused packed red cells (see donor lane in Fig. 1B).

To study the course of the disease, blood samples were taken at least every third day, beginning on Day 9 after the onset of the fever. We found the donor’s banding pattern in every blood sample. Only in the first sample, taken on Day 9 after the onset of the fever, did we detect some additional bands (Fig. 1B). These bands derive from the patient herself. The band patterns are inherited according to Mendelian laws (see parental patterns in Fig. 1B). Two days later and thereafter, the patient’s own PBLs can no longer be demonstrated. In a control study, nonfiltered blood was administered to patients who underwent open heart surgery; they did not show symptoms of TA-GVHD. By genetic fingerprinting, no donor white cells...
could be detected in the patients' PBLs in the period from 3 hours to 10 days after transfusion. Thus, even at these early time points, the recipient cells predominated over donor cells by significantly more than 100:1. As revealed in previous in vitro DNA mixing experiments (Kunstmann E. Unpublished observations, 1992) under optimal conditions (depending on the distribution of the bands and their intensities), dilutions of 1 in 40 (1 in 80) can still be detected.

After the patient's death, we examined various tissue samples (lung, spleen, skin, and lymph node) by genetic fingerprinting. Bands corresponding to the blood donor's pattern were not observed in any tissue studied (Fig. 2), which indicated less than 10 percent donor cells. Only the large fragments of skin and lymph node seem to show a difference between the tissues. This is, however, due to an electrophoretic artifact resulting from different salt concentrations in the two samples.

In addition, we investigated the lengths of the polymorphic simple repeat \((gt)_{n}\) locus in the 2nd intron of the HLA-DRB genes.\(^4\) This analysis confirmed the results obtained from the genetic fingerprints described above. The pattern of the amplified exon/intron fragment obtained from PBLs circulating in the patient differs from that of the other tissues but is identical with that of the blood donor. Even with this very sensitive method, we were unable to identify any DNA fragments originating from the donor in the tissues of the patient (Fig. 3). These results were confirmed by electroblot hybridizations (Kunstmann E. Unpublished observations, 1992; data not shown).

Discussion

In TA-GVHD, mortality is approximately 90 percent. Several technical and clinical problems make rapid diagnosis of the syndrome difficult. Clinically, single symptoms may be misinterpreted if they are not evaluated in the whole context, as the rarity of the syndrome renders its recognition more delicate, while the rapid progression of the disease makes a complete differential diagnosis difficult. Finally, the diagnosis of TA-GVHD by HLA typing is not always possible, for example, if the amount of PBLs is too low.

The examination of multilocus fingerprints via simple repetitive sequences in the genome is an alternative to the use of conventional HLA typing to make a diagnosis of TA-GVHD. With this method, any two individuals, with the exception of monozygotic twins, can be discriminated with virtual certainty. Thus, a diagnosis of TA-GVHD must be suspected if the specific banding pattern obtained from circulating PBLs differs from that obtained from tissue cells (e.g., hair root cells).

This method offers several advantages over traditional HLA typing. It can be applied even if PBL counts are low, as 100 to 250 ng of DNA, prepared from about \(4 \times 10^4\) nucleated cells, is sufficient for one genetic fingerprint. The patient's own hybridization pattern can be established without difficulty. The \((CAC)_{2}/(GTG)_{2}\) fingerprint patterns show somatic stability\(^1\) in normal human tissues. Thus, for example, hair roots can be taken for assessment of autochthon DNA without trouble to the patient. If a skin biopsy is taken for diagnosis of exanthema, DNA can also be extracted from this tissue. Furthermore, the cells used for the investigation need not be intact. DNA is in general comparatively stable, whereas degraded protein structures may not allow examination for HLA determination. Moreover, in many cases the HLA phenotype of the patient can be reconstructed only by typing the parents and siblings, as HLA typing of skin fibroblasts requires the cells to be cultured for at least 3 weeks. Yet, family members are not always available. Finally, multilocus fingerprinting is technically simple. A potential disadvantage of this method, however, is that the results are not available for 2 to 3 days, as the duration of gel electrophoresis (at least 12 hours) is a limiting factor.

Fig. 2. Hybridization patterns with the probe \((GTG)_{2}\) in \(Hinfl\)-digested DNA of the patient's tissues (t): lung (t1), spleen (t2), skin (t3), and lymph node (t4); patterns in tissue from her mother (m), her father (f), and the blood donor (d) are also shown. Note that no bands of the blood donor can be found in any tissue of the patient.
In principle, one can obtain an indication for the presence of allogenic circulating lymphocytes only 1 day after taking the blood sample. At first, DNA from PBLs is amplified by PCR and analyzed by gel electrophoresis. We routinely purify the bands by elution, label them, and separate the DNA in a denaturing, sequencing gel. This procedure also takes 2 to 3 days but has the advantage of higher sensitivity. The provisional results should be verified by specific hybridization after electrophoresing, which is possible 3 days after the beginning of the investigation. Alternatively, hypervariable DNA loci could be amplified, and the variability of these loci can be demonstrated in principle by agarose gel electrophoresis within 1 day of taking the PBLs. In the present case, we were unable to detect invading donor lymphocytes in the host organs, either by genetic fingerprinting (Fig. 2) or with the help of PCR-amplified simple repetitive sequences (Fig. 3). This means that there was less than 0.1 percent donor cells in the patient’s tissues, an amount not detectable in one round of PCR without separation of the different targets. We purposely avoided selective or exclusive amplification of donor alleles in order to generate comparative data.

It is mandatory to prevent TA-GVHD. The prognosis is fatal in most cases and no therapeutic regimen has yet been successful. Irradiation of blood components is not always practicable because of the availability of irradiators at each organization and high costs. But, contrary to expectations, white cell reduction in blood components by filtration is obviously not a sufficient prevention, as exemplified by the reported case here.

Acknowledgments
The authors are grateful to Dr. Horst Wagner for sharing clinical data; to Dr. Marcel Heim for HLA typing; to Ronald Kuehnlein for help in the preparation of the manuscript; and to Dr. Chris Linington for improving the wording and grammar.

References


