immediately and are subsequently removed by the reticular endothelial system. The different environmental conditions, required by both treatment regimens to activate p53 and CD95, may indicate that lymphocyte apoptosis induced by these mechanisms involves a different phase. With regard to UVA-induced apoptosis, these 2 phases have previously been termed immediate- and delayed-type apoptosis. The beneficial, clinical effects of ECP are thought not only to be due to the apoptosis induced in treated lymphocytes. ECP is believed also to induce a “vaccination-type” immunomodulatory response, whereby nontreated, but clonal T cells are also removed. In patients with cutaneous T-cell lymphoma (CTCL), ECP reduces the number of malignant CD4+ T cells in “responders” while CD8+ levels remain constant, a response we have also observed in patients with CTCL treated at Rotherham Photopheresis unit (P.T., unpublished data, December 2001). But in vivo chemotherapy causes a pan–T-cell depletion and lymphopenia. This difference may provide further evidence that the ECP effect is not purely linked to the induction of T-cell apoptosis. Monocytes do not become apoptotic following ECP. Conversely, ECP-treated monocytes demonstrate increased secretion of TNF-α, enhanced ability to phagocytose apoptotic T cells, and unlike with other UV therapies, retain the adhesion markers required for the presentation of processed antigens to T cells (J.B., F.T., manuscript submitted, November 2001).

The similar mechanisms, but differing outcomes, of the apoptosis induced by in vivo chemotherapy and ECP may help reinforce the importance of the monocytes in this immunomodulatory response. Further research may center on the manipulation of these antigen-presenting cells as a useful tool in the treatment of other clonal conditions.

To the editor:

Multiple myeloma: illegitimate switch recombinations and their relation to chromosomal translocations

We read with interest the article by Ho et al on illegitimate switch recombinations in multiple myeloma (MM). In this study a Southern blot method, established by Bergsagel et al, was applied to detect rearrangements in immunoglobulin heavy chain (IgH) switch regions. Five pairs of probes that hybridize upstream or downstream of each switch region were used on restriction digests of enzymes with digestion sites outside the pair of switch probes. Based on the hybridization characteristics of these probes legitimate and illegitimate switch recombination events can be distinguished; the latter may be indicative of chromosomal translocations. This assay was utilized by the authors to establish the frequency of illegitimate rearrangements as an indicator of chromosomal translocations in MM. We would like to make the following comments:

1. As initially described the appropriate restriction enzyme digests were used for the investigation of the individual switch regions (ie, HindIII for switch (S) κ and Sγ, Spil for Sμ, Sα, and Se, and BglII for Sκ, Sεμ, and Sεβ). In addition, other combinations of these enzymes and probes (BglII/Sκ, BglII/Sγ, and Spil/Sγ only the hybridization patterns of the 3′Sκ and 3′Sγ probe are shown) were applied in this study to confirm the (il)legitimate nature of single rearranging bands in more than 1 enzyme. Not all of these combinations may be suitable for the investigation of individual switch regions in terms of large-scale screening; the pairs of switch probes were initially designed to recognize the same germline fragment(s); otherwise, in the case of restriction sites between both probes, the conclusiveness of the assay is limited. With respect to the Sγ regions on BglII digests, our own results showed concomitantly as well as nonconcomitantly bands detected by the 5′ and 3′Sγ probe, respectively, suggesting BglII digestion sites in at least 1 Sγ region. Thus, it should be shown whether or not the corresponding 3′ and 5′ probes recognize the same germline fragments in the above-mentioned combinations; if not, some legitimate rearrangements might be misinterpreted as illegitimate due to the separation of the corresponding 3′ and 5′ regions.

2. Figure 2A of Ho et al shows a 4.9 kilobase pair (kbp) Spil fragment detected by both the 3′ and 5′ Sα probe that is interpreted as a translocation upstream of 5′Sα or downstream of 3′Sα. As confirmation of this rearrangement, a 5.2 kbp BglII fragment is presented that hybridizes with the 3′Sα probe but not the 5′Sα probe. If BglII encompasses both 5′ and 3′Sα regions (a prerequisite for an enzyme being used in this assay), the hybridization characteristics of the Spil and BglII fragments are mutually

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**References**

exclusive because the BgII rearrangement is detected by only 1 of the Sα probes. Therefore, either the fragments are independent of each other or there is a BgII site within this Sα region, meaning that both rearrangements may be due to the same recombination/translocation outside of this Sα region. In any case, it should not be concluded that a translocation in switch α has been confirmed by 2 restriction enzymes.

(3) Figure 2A of Ho et al1 shows 9.4 kbp and 2.0 kbp BgIII fragments detected by the 5′α probe. The latter was also detected by the 5′Sα probe. In addition, 10 and 6.5 kbp HindIII bands hybridizing with the 5′α probe are presented as confirmation of the former illegitimate rearrangements. The 2.0 kbp BgII rearrangement detected by the 5′α and 5′Sα probe strongly suggests a recombination event downstream of the 5′Sα region. The combination HindIII/5′α, however, covers the region upstream of 5′Sα including the joining region (Jh). Thus the 2.0 kbp BgII fragment must be independent of the HindIII fragments mentioned. Alternatively, these HindIII rearrangements could be due to VDJ recombination events, which should have been tested with a Jh probe (Bergsagel et al2[Pe2] for restriction map and positions of probes).

(4) In Figure 2A,C Ho et al1 show 12 kbp and 3.5 kbp HindIII fragments detected by the 3′Sγ probe. These rearrangements were designated illegitimate recombination events as they were not detected by probes for upstream or downstream acceptor sites. However it is not mentioned whether or not these fragments hybridized with the 5′Sγ probe. This information is crucial to exclude known HindIII restriction fragment length polymorphisms (RFLPs) of the switch γ region.2,4

In summary we wish to point out that the true nature of IgH-associated rearrangements remains obscure until the fragments have been cloned. Illegitimate rearrangements are indicative of, but do not necessarily prove, chromosomal translocations; they may also be due to polymorphisms and intrachromosomal rearrangements.2 Thus in terms of large-scale screening the true frequency of translocations may be overestimated. In our experience the choice of suitable alternative restriction enzymes for confirmation of single IgH-associated (il)legitimate rearrangements has been complicated by the high frequency of RFLPs in certain enzyme digests, digestion sites within switch regions, as well as unavailability of reliable DNA sequence data for restriction mapping. If, in the context of the above-mentioned assay, alternative restriction enzyme combinations are used for the investigation of switch regions, additional information should be given as to whether or not the corresponding 3′ and 5′ switch probes recognize the same germline fragment. In any case the results should be interpreted with care.

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References


Response:

Illegitimate switch recombinations in primary myeloma tumor

We thank Dr Schmidt for his comments on our study of illegitimate IgH switch recombinations in primary myeloma tumor.1 The first concern the letter raises relates to our use of combinations of switch probes and restriction enzymes “alternative” to those originally designed to hybridize the 5′ and 3′ ends of the switch regions, with restriction sites located outside the probes (HindIII – 5′Sα, 3′Sα, 5′Sγ, 3′Sγ; SphI – 5′Sμ, 3′Sμ, 5′Sα, 3′Sα, 5′Sε, 3′Sε; and BgII – 5′Sμ, 3′Sμ, 5′α, 3′α).2 First, we wish to emphasize (as stated in “Materials and methods”)1 that the strategy by which we screened patients for illegitimate recombinations, and by which all recombinant bands designated as illegitimate were initially detected, utilized the above enzyme/probe combinations. We agree that cloning would be most accurate in determining the nature of these fragments. However, whereas myeloma cell lines (from which the majority of translocation breakpoints have been cloned) and other lymphoid tumor biopsies may provide abundant DNA for cloning, cell numbers in marrow biopsies are often very limited. We therefore adopted the approach of determining whether a recombinant fragment was legitimate or illegitimate by “matching” with other switch probes. As stated in “Materials and methods,”1 Southern hybridizations were performed with alternative enzyme/probe combinations, which had previously revealed recombinant fragments during screening. The basis of this approach was that if a fragment resulted from a legitimate recombination between 2 switch regions and was detected by 1 of the 2 switch probes, then a matching fragment should be detected by the other probe, provided that there are no restriction sites internal to the probes. From the sequence data available, we confirmed that there are no such internal restriction sites for HindIII – 5′Sα, 3′Sα; SphI – 3′Sγ; and BgII – 5′Sα, 3′Sα that were required for our analysis. For the remaining combination BgIII-3′Sγ, there are no BgII sites in Sγ2, 3, and 4, but 1 site is present in Sγ1. In our cohort, BgIII-3′Sγ hybridization was performed to verify the nature of nongermline BgIII bands detected by Sμ probes in samples also demonstrating HindIII-3′Sγ nongermline fragments. The difficulty in interpreting these BgII digests occurs when a legitimate Sμ to Sγ1 switch may be considered illegitimate due to BgII digestion in Sγ1. For all cases in which BgII-3′Sγ hybridization was done to “match” with BgII-Sμ fragments, we emphasized the following: (1) HindIII-5′Sμ, 3′Sμ, and 3′Sγ were also performed; (2) in all of these cases but 1 (Patient 1 discussed below), we interpreted the results of both enzymes such that no BgII-Sμ fragment was deemed illegitimate without confirmation with HindIII; (3) and BgII-3′Sγ remains informative for the other 3 Sγ regions. As we discussed in the paper, we are fully aware that some unmatched fragments could have resulted from polymorphisms, internal rearrangements, and
deletions. We would like to point out that in each hybridization we can detect the germline band(s) of expected size, either from the nontumor cells in the marrow sample or the nontranslocated allele, serving as an additional internal control. Importantly, alternative enzyme/probe hybridizations were performed in our study according to recombinant bands already demonstrated with the “original” combinations and were used primarily as confirmation of the screening Southern blots. These alternative combinations were useful in eliminating undetected legitimate (especially downstream and inversion) switches, and possible artifacts that can be introduced by restriction fragment length polymorphisms (RFLPs) even in the original screening hybridizations.

The second criticism of Dr Schmidt relates to our analysis of 1 of 4 examples in Figure 2A (Patient 1). We agree that if a single translocation accounts for the 4.9 kilobase (kb) SphI fragments detected by 5'Sαa and 3'Sαa, and the 5.2 kb BglII-3'Sαa fragment, then a BglII recombinant band should also be detected by 5'Sαa. However BglII-3'Sαa is clearly germline. From the sequence database, we verified for both ScI and 2 that a BgII site is present 1.3 kb downstream of the 3'S probe (292 base pair upstream of the 3'SphI site), and there are no other BglII sites in Sαa. From our germline hybridization, upstream BgII sites appear to be located at least 12 kb from the 3'S site. Hence the 5.2 kb BglII fragment detected by 3'Sαa is most likely to be caused by an extraneous BglII site on a translocated fragment at the 5’ end, replacing the 5'Sαa probe sequence. In the absence of other BglII sites in Sαa, we agree with Dr Schmidt that based on our findings, the SphI-5’Sαa and 3’Sαa recombinant fragments, although of the same size, must be independent. However this does not reduce the possibility as we had suggested that the 5.2 kb BglII and the 4.9 kb SphI 3’Sαa fragments most likely represent the same illegitimate recombination; although this could only be formally proved by cloning. We have repeatedly demonstrated the SphI-5’Sαa fragment and the absence of matching nongermline SphI fragments hybridized by 3’Sαy and 5’Sαa, excluding downstream or inversion switches. We accept the criticism that the HindIII 3’Sμa recombinant fragments cannot be used as confirmation of the 9.4 kb BgII fragment, as 5’Sμa is located upstream of the HindIII site, a fact that we had specified in the legend to Figure 2A(II) but overlooked in the analysis. Nevertheless Dr Schmidt agrees with us that the 2 kb BgII fragments hybridized by both 5’Sμa and 5’Sμa strongly suggest a recombination event downstream of 5’Sμa. From this analysis we believe that our data remain fully consistent with the presence of illegitimate recombinations in this patient, and our conclusions on the relationship with disease behavior are unchanged. Regarding HindIII RFLPs in Sγa, we wish to point out that HindIII digests from all patients were hybridized by both 5’Sγa and 3’Sγa, which would have shown if any of the extraneous bands were due to RFLPs. For the 12 kb and 3.5 kb HindIII-3’Sγa bands(1H[FIG 2A(C)]) we can confirm that no such bands were detected by 5’Sγa.

As a result, while cloning provides the ultimate proof of the nature of a recombinant fragment in the IgH genes, we believe that our investigation of illegitimate switch recombinations by the adaptation of an established Southern Blot assay was justified, given the paucity of available tumor material from human myeloma bone marrow. Our exhaustive analysis using the original screening blots for detection of possible illegitimate switches and their verification by probes and enzymes that have previously revealed recombinant bands have provided us with useful and reliable information. Since our paper was published more than a year ago, substantial refinements have been made to molecular cytogenetics, which are likely to be quicker, less labor intensive and more accurate in demonstrating chromosome 14q translocations, and would be superior to Southern hybridization for large-scale patient screening. Finally, we would like to re-emphasize that our conclusions regarding illegitimate switch recombinations were made after careful investigation of each nongermline band. We fully agree that detailed analysis is required in the interpretation of the Southern Blot assay and thank Dr Schmidt for his note of caution.

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To the editor:

Myelodysplastic syndromes: from French-American-British to World Health Organization: a commentary

Nösslinger and coworkers1 are to be complimented on a carefully conducted retrospective survival study of 431 patients with primary myelodysplastic syndromes (MDSs) comparing the original French-American-British (FAB) proposals2 and the recently published World Health Organization (WHO) proposal classifications of MDS.3 However, we are concerned about the authors’ interpretation of the WHO criteria and the resulting impact on their survival studies. The critical changes in the WHO classification from the FAB include the following: (1) lowering the blast percentage for the diagnosis of acute myeloid leukemia (AML) to 20% from 30%, thus eliminating refractory anemia with excess blasts in transformation (RAEB-T); (2) moving dysplastic chronic myelomonocytic leukemia (CMMML) into a proposed new category of myeloid disease with features overlapping myelodysplastic syndromes and myeloproliferative disorders; (3) subdividing RAEB into 2 types: RAEB-1 (5%-9% marrow blasts) and RAEB-2 (10%-19% marrow blasts); and (4) separating refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) into 2 broad categories

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