Expression of p53, p21/wafl , bcl-2, bax, Rb and Ki67 proteins in Hodgkin's lymphomas

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Summary. The aim was to investigate the combined immunoexpression of p53, p21, bcl-2, bax, Rb and Ki67 proteins in Hodgkin's lymphomas (HL) and correlate expression patterns with the histotype and the Epstein-Barr Virus (EBV) status. Paraffin-sections from 56 cases of HL (18 nodular sclerosis and 38 mixed cellularity) and from ten "reactive" lymph nodes were investigated. P53, p21, bcl-2, bax, Rb and Ki67 proteins were detected in Hodgkin and Reed-Sternberg (HRS) cells in 35156,56156,24156, 23156,56156 and 56/56 cases of HL, respectively. No correlation was found between the expression of each protein and the EBV status or the histotype of HL. Comparison between p53 and p21 staining revealed two patterns: a) p53+/p21+ (35 cases); and b) p53-/p21+ (21 cases). The pattern p53+/p21+ suggests wild type p53 protein able to induce the expression of p21 while the p53-/p21+ pattern suggests p53-independent p21 expression. These results are consistent with the interpretation that inactivating p53 gene mutations may be rare in HL. Comparison between bcl-2 and bax staining showed a statistically significant relationship (p<0.001) for coexpression (19 cases) or absence of expression of both proteins (28 cases) in HRS cells. In contrast, bax expression was observed in most lymphoid cells in all "reactive" lymph nodes. Since the proapoptotic bax protein may act as tumour suppressor it is possible that the absence of this protein in HRS cells in a substantial proportion of HL may confer growth advantage and play a role in their pathogenesis. This could suggest bax gene alterations in some HL since in other studies acute lymphoblastic leukaemia cell lines demonstrate bax gene mutations with loss of bax immunoexpression. Another possibility is that reduced bax expression may be due to post transcriptional regulation, as was described in lymphoma cell lines. Comparison between Rb and Ki67 staining disclosed two main deviations from the normal parallel relationship in reactive lymph nodes: a) 2 cases with low

Rb and high Ki67 expression possibly reflecting loss of Rb expression due to chromosome loss or to other abnormalities in the structure or the expression of Rb gene; and b) 9 cases with high RB and low Ki67 possible reflecting an attempt of Rb protein in excess to induce cell cycle arrest. Taken together, our findings provide combined immunohistological evidence for deregulated expression of cell-cycle and apoptosis-related proteins, that may play a role in the pathogenesis of HL.

Key words: Hodgkin's lymphomas, Immunohisto-chemistry, Apoptosis, Cell 'cycle

Introduction

Despite the enormous variations in human tumour types a common theme in oncogenesis is the perturbation of the complex network proliferation/cell-cycle arrest/differentiation/apoptosis. Analysis of the proteins involved in this network should help to clarify the multiple pathways of oncogenesis. p53 has a central role in the regulation of the cell-cycle and the apoptosis (Miyashita and Reed, 1994; Cox, 1997; Morente et al., 1997; Evan and Littlewood, 1998; Prives and Hall, 1999). There is evidence that the levels of p53 a) are regulated by its interaction with mdm2, b) are modified after cellular stress and c) are altered by a stress signal independent pathway in which the product of the alternative reading frame (ARF) of the P16 locus is induced and this leads to abrogation of mdm2-targeted destabilization of p53 (Prives and Hall, 1999). Wild-type (wt) p53 transcriptionally upregulates p21/waf1 protein which inactivates the cyclin/cyclin-dependent-kinase (CDK) complexes resulting in inhibition of Retinoblastoma (Rb) protein phosphorylation and blocking the cell-cycle progression in the G1-S transition (El Deiry et al., 1993; Cordon-Cardo and Richon, 1994; Waga et al., 1994; Elledge, 1996, Cox, 1997; Prives and Hall, 1999). In addition, p21/waf1 protein blocks the action of proliferating cell nuclear antigen (PCNA), impeding DNA replication (Waga et

al., 1994). Inactivation of p21 could result in abnormal DNA replication control or loss of coordination between DNA replication and the cell-cycle progression (Xiong et al., 1993; Waga et al., 1994). Both could lead to genome instability which is directly related to oncogenesis. On the other hand, p21 expression can also be regulated independently of p53 expression (Steinmann et al., 1994; Cox, 1997). Interestingly, whereas mutations of the p53 gene are the most common genetic abnormalities in neoplasia (Greenblat et al., 1994), those of waf-1 gene which codes for the p21 protein are mostly absent in human tumours (Shiohara et al., 1994).

Besides cell-cycle arrest, p53 is involved in apoptosis and a recent study (Polyak et al., 1997) suggested that p53 results in apoptosis through a three-step process: a) the transcriptional induction of redox-related genes; b) the formation of reactive oxygen species; and c) the oxidative degradation of mitochondrial components. p53 has also been reported to influence the expression of the bcl-2 and bax genes (Miyashita et al., 1994; Miyashita and Reed, 1995) which are involved in the regulation of apoptosis (Cox, 1997; Save et al., 1998). There is evidence that p53 downregulates the expression of the anti-apoptotic bcl-2 gene and upregulates the expression of bax which is an inducer of apoptosis (Miyashita et al., 1994; Miyashita and Reed, 1995; Adams and Cory, 1998). Of interest, and it has been suggested that bax/bcl-2 and bax/bax protein dimerizations may be associated with cell survival and cell death, respectively (Adams and Cory, 1998). It has been suggested that the pro-apoptotic effects of bax may be elicited through an intrinsic pore-forming activity that can be antagonized by bcl-2 (Adams and Cory, 1998). In addition, there is evidence that bax acts as a tumour suppressor gene (Yin et al., 1997). Indeed, transgenic mice that express a truncated T antigen that inhibits Rb but leaves p53 intact displayed an accelerated progression to malignancy upon a bax-deficient background (Yin et al., 1997). Previous studies have described the involvement of the bcl-2 and bax proteins in lymphoid malignancies (Doussis et al., 1993; Brousset et al., 1996; Leoncini et al., 1997).

p53 has also been reported to negatively regulate the expression of retinoblastoma (Rb) tumour suppressor gene (Shiio et al., 1992). The Rb gene product is active in growth suppression during the G0 and G1 phases and is then phosphorylated and rendered inactive for the remainder of the cell cycle (Wiman, 1993; Evan and Littlewood, 1998; Messineo et al., 1998). Abnormalities in the structure and/or the expression of the Rb gene may inactivate its growth suppressive function and these have been described in lymphoid tumours (Wiman, 1993; Sanchez-Beato et al., 1996a; Morente et al., 1997).

HL have been extensively studied for the expression of many cell-cycle proteins, oncogenes and tumour suppressor genes (Dousssis et al., 1993; Jiwa et al., 1993; Zhu et al., 1995; Brousset et al., 1996; Sanchez-Beato et al 1996a,b; Leoncini et al., 1997; Lorenzen et

al., 1997; Morente et al., 1997). However, there is yet no combined immunohistological information regarding the expression of p53 in relation with that of the p53-influenced p21, Rb, bcl-2 and bax proteins and with the expression of Ki67 protein in a large series of these tumours. This study was undertaken in order to investigate the combined expression of the afore-mentioned proteins in 56 cases of HL and to correlate the immunoprofiles with the histotypes of HL. Moreover, the expression patterns were compared to the EBV status as determined by EBER1/2 RNA in situ hybridisation (RISH) and LMP-1 immunohisto-chemistry, since the levels of expression of Rb, p53, p21 and bcl2 proteins are modulated in vitro by EBV infection of B-cells (Cannell et al., 1996) and there are also other important interactions between EBV and the cell cycle control network (Sinclair et al., 1998).

Materials and methods

The material comprised of 56 cases of HL (18 of nodular sclerosis and 38 of mixed cellularity) (Harris et al., 1994) which were retrieved from the files of the Pathology Departments of the University Hospital of Heraklion, Venizelion Hospital of Heraklion and Evagelismos Hospital of Athens during the period 1992-1998. In addition, 10 "reactive" lymph nodes were studied as control.

Immunohistochemistry and RNA in situ hybridization

Immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections, using the alkaline phosphatase (APAAP) method. The anti-p53 Do7 (Dako) (Glostrup, Denmark), the anti-p21 (EA10) (Oncogene Sciences) (Cambridge MA, USA), the anti-bax (Immunotech) (Marseille, France), the MIBI Ki67 (Immunotech), the Rb1 (Dako), the LMP-1 (Dako) and the anti-BCL2 (124) (Dako) monoclonal antibodies were used. The bridging rabbit antimouse (Z 259) and APAAP complexes (D 651) were obtained from Dako. A step of microwave heating in a solution of sodium citrate was performed prior to incubation with all antibodies. Briefly, xylene dewaxed and alcohol rehydrated paraffin wax sections were placed in coplin jars filled with a 0.01M tri-sodium citrate solution, and heated three times in a conventional microwave oven for five minutes at 700W. After microwave processing, slides were allowed to cool at room temperature for 15 minutes. They were then washed in Tris-buffered saline (TBS), pH 7.4, and incubated with the specific antibody. Positive control slides were included in all tests and consisted of paraffin sections from our previous study (Stefanaki et al., 1997) known to be positive for p53, p21/waf1, Rb, Ki67, bax and bcl-2. For MIB1 (Ki67), Rb, bcl-2 and bax internal positive control was required. Negative control slides were prepared by omitting the primary antibody. Cases were divided into groups according to the positivity of HRS cells for p53, p21, Ki67, Rb, bcl-2 and bax

expression (<10%, 10-25%, >25%). Nuclear expression was considered as positive for p53, p21, Ki67 and Rb proteins and cytoplasmic expression as positive for bcl-2 and bax proteins. The protein immunoexpression was counted in 20 selected fields with numerous positive cells at x40 magnification. RNA in situ hybridiration for the detection of EBV-encoded EBER1/2 mRNAs was performed as we described previously using oligonucleotide EBER probes (Dako) (Kanavaros et al., 1993, 1994). Positive EBV control were cases of Hodgkin's lymphomas that we described previously (Kanavaros et al., 1994).

Statistically analysis

Statistical analysis was performed with SPSS program for Windows (6.0, 1993), using Chi-square test. Fisher's test was used when there were less than 5 cases in one group included in a table for statistical evaluation. The results were considered as statistically significant when Pearson's correlation coefficient showed $p<0.05$, and highly significant when $p<0,01$.

Results

The staining results for antibodies and EBER 1/2 probes in HRS cells and the combined immunophenotypes are summarized in Table 1-5. p53 nuclear expression was restricted in HRS cells (Fig. 1). Rb and p21 nuclear expression was found in HRS cells and in small lymphocytes, but p21-positive small cells were very rare. Bcl-2 and bax cytoplasmic expression was evidenced in HRS cells and small lymphocytes.

p53, bcl-2 and bax were completely undetectable in HRS cells in 21/56, 32/56, and 33/56 cases, respectively. Ki67 (Fig. 2), p21 and Rb expression (Fig. 3) was found in HRS cells in all cases (ranging from very few HRS cells to more than 25% of HRS cells).

No correlation was found between the expression of p53, p21, Rb, Ki67, bcl-2 and bax proteins with the histotypes of HL (Table 1) or the EBV status.

In all ten "reactive" lymph nodes numerous Rb-positive cells (10-25%) paralleled Ki67-positive cells in germinal centers and interfollicular areas (Cannell et al., 1996). p53- and p21-positive cells were absent or very

Table 1. Immunophenotypes of HRS cells in relationship with the histotypes of HL.

	P53	P21	Rb	Ki67	Bcl-2	Bax
NS	9/18	18/18	18/18	18/18	5/18	7/18
MC	26/38	38/38	38/38	38/38	19/38	16/38
Total	35/56	56/56	56/56	56/56	24/56	23/56

Table 2. EBV status in HRS cells in relationship with the histotypes of HL.

	EBER	LMP-1
NS	2/18	2/18
MC	15/38	15/38
Total	17/38	17/38
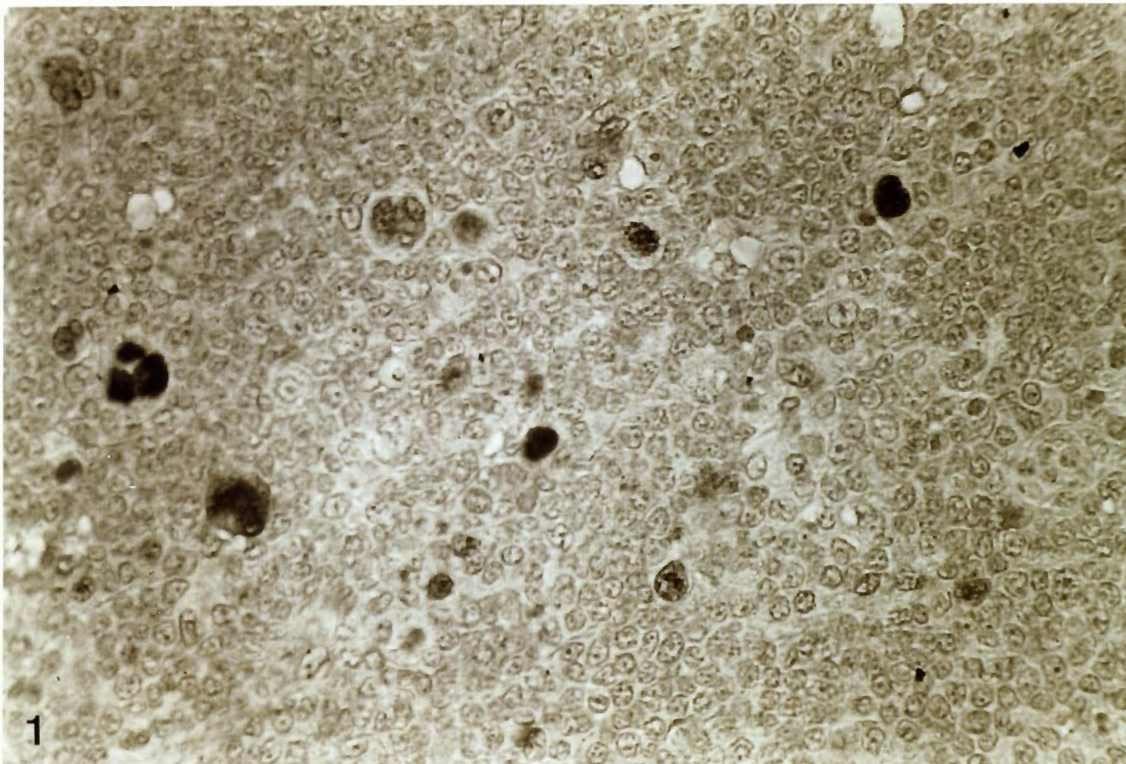
Fig. 1. Expression of p53 protein in HRS cells of HL. x 400

Table 3. Combined immunoprofiles of Ki67, Rb, p53 and p21 in HRS cells.

Ki67/Rb Ki67	Rb <10%	10-25%	>25%
<10%	3	17	9
10-25%	2	1	6
>25%	2	13	3

Ki67/p21 Ki67	p21 <10%	10-25%	>25%
<10%	5	18	6
10-25%	2	3	4
>25%	2	11	5

Ki67/p53 Ki67	p53 <10%	10-25%	>25%
<10%	17	1	0
10-25%	7	3	0
>25%	3	4	0

P53/p21 p53	p21 <10%	10-25%	>25%
<10%	11	13	3
10-25%	1	6	1
>25%	0	0	0

P53/Rb p53	Rb <10%	10-25%	>25%
<10%	2	17	8
10-25%	5	2	1
>25%	0	0	0

rare (<1%) in 7 cases and localised in the same areas (Mateo et al., 1997). Numerous bax-positive cells were found in germinal centres and interfollicular areas in all cases of "reactive" lymph nodes. Bcl-2 stained mantle roue and interfollicular areas while germinal centres were mainly negative.

Relationship between p53/p21, p53/bcl-2, p53/bax and p53/Rb expression

Since p53 can influence the expression of p21, bcl-2, bax and Rb the corresponding immunoprofiles in HRS cells were determined.

No statistically significant coexpression or inverse expression was found between p53/Rb or p53/bcl-2 or p53/bax or p53/p21 proteins.

Relationship between bcl-2 and bax expression

A statistically significant (p<0.001) coexpression or absence of expression of both proteins was found (Tables 4 and 5). In contrast, there was no statistically significant relationship between each of the patterns of the Table 5 and the histotype or the EBV status.

Relationship between Ki67/Rb, Ki67/p21 and Ki67/p53 expression (Table 3)

No statistically significant coexpression or inverse expression was found between Ki67/Rb, Ki67/p21 or Ki67/p53.
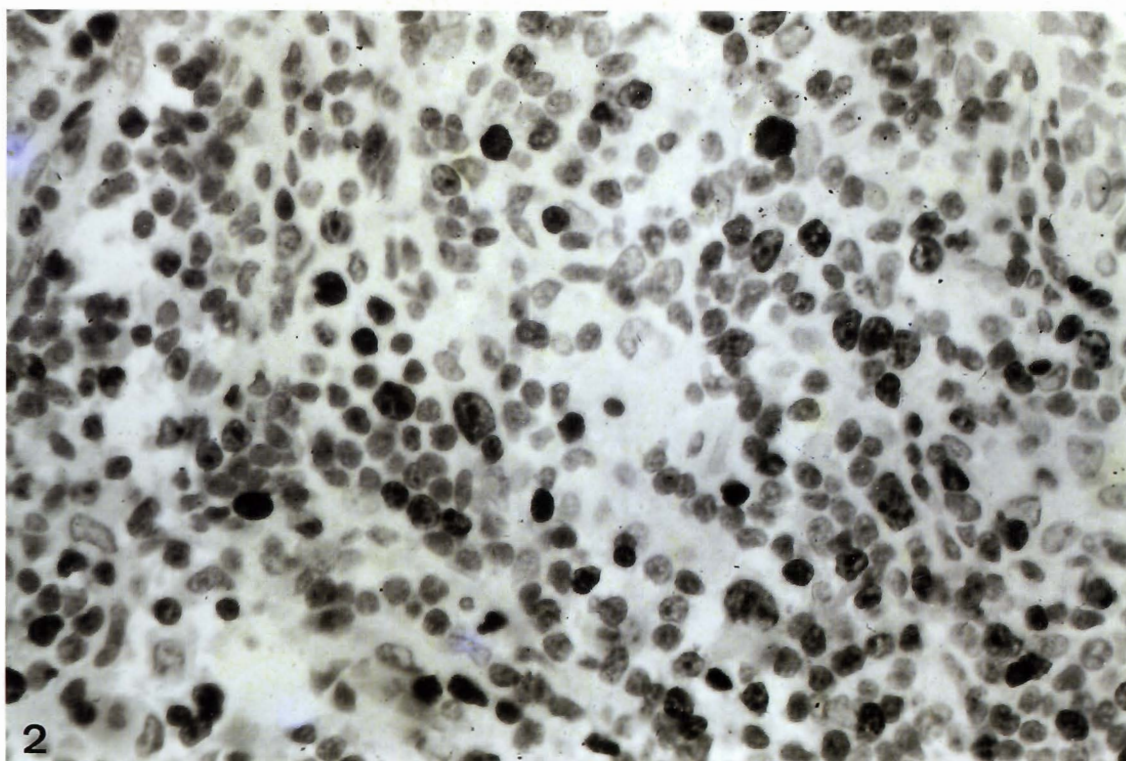

Fig 2. Expression of MIB1 (Ki67) in many cells in HL. x 400

Discussion

In our study we have attempted to determine the immunohistological relationships between p53 expression and those of p21, bcl-2, bax, Rb and Ki67 in order to gain insight into some in-situ aspects of cell-cycle and apoptosis in HL.

1) Relationship between p53 and p21 expression

In our study 35/56 cases of HL showed p53

expression in HRS cells and in 8 of them more than 10% of HRS cells were p53 positive. However, it seems unlikely that this expression reflects underlying p53 gene mutations as there is evidence for absence of p53 gene mutations in most HL (Xerri et al., 1995) although the possibility of mutations occurring outside the classic "hot-spots" cannot be excluded (Kocialkowki et al., 1995). As an indirect approach to evaluate the p53 gene status, the expression of p21 has been studied, since p21 expression may be induced by wt p53 (Cordon-Cardo

Table 4. Combined immunoprofiles bcl2, Bax and p53 in HRS cells.

Bcl-2/p53 Bcl-2	p53 <10%	10-25%	>25%
<10%	4	3	0
10-25%	6	3	0
>25%	2	0	0
Bax/p53 Bax	**p53 <10%**	**10-25%**	**>25%**
<10%	2	3	0
10-25%	4	2	0
>25%	1	0	0
Bcl-2/Bax Bcl-2	**Bax <10%**	**10-25%**	**>25%**
<10%	6	6	2
10-25%	5	0	0
>25%	1	0	1

Table 5. Patterns of bcl2/p53, Bax/p53 and bcl2/Bax expression in HRS cells.

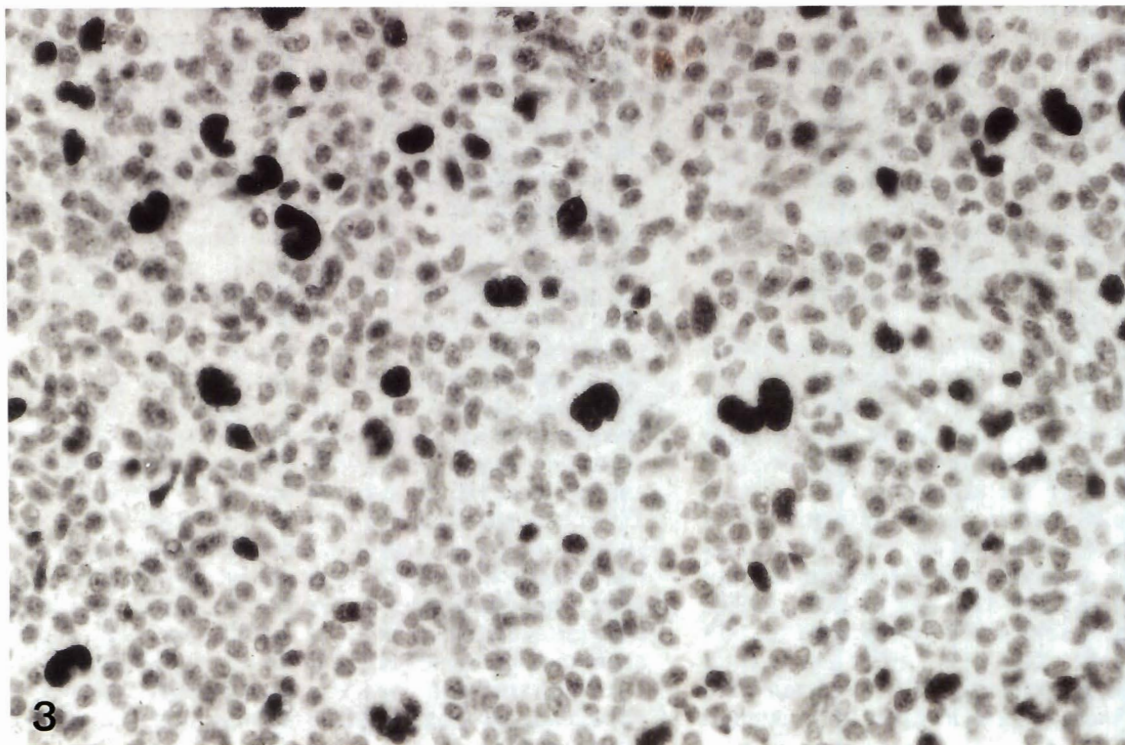| Bcl-2/p53 Bcl-2	p53 +	-	Total
+	18	6	24
-	17	15	32
Total	35	21	
Bax/p53 Bax	**p53 +**	**-**	**Total**
+	12	11	23
-	23	10	33
Total	35	21	
Bcl-2/Bax Bcl-2	**Bax +**	**-**	**Total**
+	19	5	24
-	4	28	32
Total	23	33	

Fig 3. Expression of Rb protein in many cells in HL. x 400

and Richon, 1994; Sanchez-Beato et al., 1996a; Villuendas et al., 1997; Prives and Hall, 1999). Thus, we have determined the p53/p21 patterns: a) The pattern p53-/p21+ (21 cases). This pattern suggests p53-independent pathway of p21 induction of expression (Steinmann et al., 1994; Cox 1997). However, negative p53 immunostaining may also result from biallelic deletion of p53 gene (Kocialkowski et al., 1995; Cox, 1997), but this seems very unlikely in most HL (Trumper et al., 1993; Xerri et al., 1995). b) The pattern p53+/p21+ (35 case). This pattern indicates wild type p53 protein able to induce p21 expression (Cox, 1997). A previous study on lymphomas (Villuendas et al., 1997) showed that the p53+/p21- phenotype was associated to p53 mutations. Interestingly, no p53+/p21- HL cases were found in our study supporting the previous evidence that p53 mutations are rare in HL (Xerri et al., 1995). However, another study (Trumper et al., 1993) demonstrated mutation of p53 gene codon 246 in 5 of 7 HRS cells examined by single cell PCR in 3/7 patients with HL. It has been suggested that p53 gene mutation may represent a late event in the pathogenesis of some HL associated with disease progression, since only a proportion of HRS cells harboured this mutation (Trumper et al., 1993). Therefore, the search for p53 mutations in HL seems to require single-cell PCR. Besides p53 gene mutations it is possible that accumulation of p53 protein could occur as a consequence of absence of mdm-2 expression, since the p53/mdm-2 interaction can result in enhanced p53 degradation (Save et al., 1998). However, the detection of mdm-2 protein and mRNA in all HL studied does not favour this interpretation (Sanchez-Beato et al., 1996b). p53 protein immunodetection could also be the result of molecular complex with the EBNA-5 protein of EBV (Szekely et al., 1993), but no correlation was found in our study between EBV status and p53 expression. In addition, there is no evidence that EBNA-5 is expressed in HRS cells of HL and it is noteworthy that EBV-induced immortalisation does not seem to inactivate the function of p53 (Cannell et al., 1996). An alternative hypothesis to explain p53 protein accumulation without mutation could be the stabilisation of wild-type p53 protein through direct association to hypoxia-inducible factor 1a (An et al., 1998)

It should be mentioned, however, that an important caveat of the immunohistochemical analysis of p53/p21 pattern for prediction of p53 activity are the data that each of p53 and p21 proteins can probably regulate more than one pathway which may be mutually exclusive (e.g apoptosis and cell-cycle arrest) (Cox, 1997). Thus, although the p53/p21 pattern can be informative in certain circumstances (Lorenzen et al., 1997; Villuendas et al., 1997), reliable assessment of p53 protein activity should be based on DNA and RNA studies (Cox, 1997). Taken together, the present and previous data on p53 expression in HRS cells (Xerri et al., 1995; Lorenzen et al., 1997), favour the interpretation of a wt protein able to upregulate p21 expression and involved in an attempt

for cell-cycle arrest. A role of p53 expression in HRS cells for apoptosis could be considered. However, these cells did not exhibit classical apoptotic features when studied by electron microscopy and the TUNNEL method although the possibility of apoptosis without typical features cannot be excluded (Lorenzen et al., 1997).

In line with previous data (Lorenzen et al., 1997; Naresh et al., 1997), many HL expressed the p21 protein in more than 10% of HRS cells in this study. Furthermore, we found no statistically significant tendency for parallel or inverse expression of Ki67 and p21 proteins. It should be noticed, however, that 5 cases showed expression of both proteins in more than 25% of HRS cells, and all were p53 immunopositive. This suggests that the presumptive p53-induced p21-mediated growth arrest is somehow overriden in this subset of HL. The inability of p21 to induce growth arrest could be related to p21 gene structural alterations but this is unlikely in most human tumours (Shiohara et al., 1994). Other possibilities could be insufficient amounts of p21 protein to inhibit cell-cycle or alterations of the p21 targets (Elledge et al., 1996).

2) Relationship between bax, bcl-2 and p53 expression

In our study bax expression was undetectable in HRS cells in 33 cases, whereas most small lymphocytes in HD as well as most normal lymphoid cells in reactive lymph nodes were bax positive. This suggests deregulated expression and could be interesting in view of recent data (Yin et al., 1997) that bax acts as tumour suppressor. The putative role of absence of bax in HL could be related to recent findings in acute lympho-blastic leukaemia cell lines that bax gene mutations resulted in absence of detectable amounts of bax protein (Meijerink et al., 1998). Thus it is possible that reduced bax expression in HRS cells may be related, at least in some cases, to underlying mutations which could confer survival advantage during the development and progression of HL. Therefore, it would be interesting to analyse possible genetic alterations of bax in HL although the one HL-cell line studied showed wild-type bax gene (Meijerink et al., 1998). Another possibility is that reduced bax expression can be due to post-transcriptional regulation of bax protein levels as described in lymphoma cell lines (Leff et al., 1996). Mention should be made, however, to some discrepancies in previous studies (Brousset et al., 1996; Messineo et al., 1998) with respect to bax immuno-expression in HL. Whereas in one study (Brousset et al., 1996) bax was found in HRS cells in most HL, in our study as well as in another study (Messineo et al., 1998) bax protein and mRNA were rarely expressed in HRS cells. This discrepancy is likely to be due to the use of polyclonal (Brousset et al., 1996) or monoclonal antibodies (Messineo et al., 1998, our study). Since non-specific staining was suggested with the use of polyclonal anti-bax antibody (Messineo et al., 1998) it

seems likely that bax expression is not frequent in HRS cells of HL. On the other hand the comparison between bax and bcl-2 expression in HRS cells of HL revealed a statistically significant tendency for absence of expression of both proteins or for coexpression. These findings suggest that a delicate balance between pro-apoptotic (bax) and anti-apoptotic stimuli (bcl-2) is retained in many HL (19 cases). Interestingly, 28 bax-negative tumours of our study were also bcl-2 negative. It could be suggested that bcl-2-negative tumours may have evolved to a point where they are less dependent on bcl-2 for their survival and that one of the reasons for this reduced dependence on bcl-2 may be the low bax expression. However, since bcl-2 is not the only anti-apoptotic member of the bcl-2 protein family (Adams and Cory, 1998), it is possible that low bax expression may be important because the ratio of bax with other anti-apoptotic proteins such as bcl-xl and mcl-1 presumably has been modified in tumours with low bax expression. In this regard previous studies (Brousset et al., 1996; Messineo et al., 1998) showed frequent immonoexpression of mcl-1 and bcl-x and frequent mRNA expression of bcl-xl (Messineo et al., 1998) in HRS cells suggesting a role of these apoptosis-related proteins in HL. The mechanisms of reduced bax expression in a subset of HL may be related to p53 since this gene can transactivate the bax gene promoter which contains four consensus binding site sequences for p53 (Miyashita and Reed, 1995). However, no statistically significant correlation was found between increased p53 and bax immunoexpression in our study. This finding could be related to previous evidence for p53-independent mechanisms for regulating bax gene expression (Miyashita et al., 1994).

Although, bcl-2 expression can be down-regulated in vitro by wild-type and mutant p53 (Adams and Cory, 1998), in agreement with previous data (Doussis et al., 1993), we found no statistically significant coexpression or inverse expression between bcl-2 and p53 in HL. This finding can also be compared with our previous data that there was an inverse bcl2/p53 relationship in thymomas (Stefanaki et al., 1997). These data suggest that bcl-2/p53 interactions may be tumour-type dependent and do not seem to be influenced by EBV infection in EBV-associated tumours such as HL.

3) Relationship between Rb, and Ki67 expression

In some cases of HL the relationship between RB and Ki67 expression has been disturbed and two main deviations from the normal pattern of the reactive lymph nodes have been observed: a) A subset of cases (2 cases) showed low (<10%) Rb protein expression in relationship with high (>25%) Ki67 expression. This could reflect loss of Rb expression due to loss of chromosome 13 since Rb gene is located in 13q14 (Wiman, 1993; Cordon-Cardo and Richon, 1994). In this respect, a previous study (Tilly et al., 1991) in untreated cases of HL showed frequent loss of chromosome 13

suggesting the possibility of loss of heterozygosity of Rb in the pathogenesis of some HL. Another possibility is Rb gene mutation or defects in the transcription and translation of Rb gene; b) Another subset of cases (9 cases) showed high Rb (>25%) with low Ki67 (<10%) expression. This could reflect an attempt of the Rb protein in excess to induce cell cycle arrest. Another hypothesis is increased Rb expression due to infection from EBV. Indeed, Rb protein can form complexes with EBNA-5 protein (Szekely et al., 1993) and EBV-transformed B-cells show increased level of hyperphosphorylated Rb protein in comparison to primary non-infected B-cells (Cannell et al., 1996). Evidence was provided that EBV uses a strategy involving hyperphosphorylation of pRb and altered expression of other important cell-cycle proteins (i.e. p21, p53, bcl-2) in order to drive cell proliferation (Cannell et al., 1996). However, in our series, no positive correlation was found between increased Rb expression and EBV status in HRS cells.

In summary, our findings provide combined immunohistological evidence for a deregulation of the expression of apoptotic and cell cycle proteins, such as bax, bcl-2, p53, p21, Rb and Ki67, that may play a role in the pathogenesis of HL.

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