Elevated Serum Transforming Growth Factor β 1 Levels in Epstein-Barr Virus-Associated Diseases and Their Correlation with Virus-Specific Immunoglobulin A (IgA) and IgM

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Transforming growth factor β (TGF- β) is an immunosuppressive cytokine which can induce immunoglobulin A (IgA) switch and Epstein-Barr virus (EBV) replication in latently infected cells. Here we report elevated serum levels of TGF- β in various EBV-associated diseases correlating positively with EBV-specific IgA titers and negatively with IgM titers, suggesting a role for this cytokine in the pathogenesis of these diseases.

The transforming growth factor β (TGF- β) family of cytokines is a group of closely related polypeptides of which TGF- β 1 is the prototype. TGF- β is one of the most immunosuppressive substances found in the human body (reviewed in reference 11). It is produced by a wide variety of cells and tissues and plays an important role in cell differentiation, growth, matrix formation, and the regulation of immune and inflammatory responses (11). It may inhibit or stimulate cell growth, depending upon the cell type and culture conditions. Receptors for TGF- β have been found almost on every cell line tested so far, which enables this cytokine to exert its effects on almost any body tissue (reviewed in reference 13). TGF- β -mediated signaling involves some unique second messengers and transcription factors called SMAD proteins. Defects in the TGF- β mediating signaling pathways have been causally linked to several human cancers (7, 12, 27).

Earlier studies have demonstrated that Epstein-Barr virus (EBV) and its gene products can induce TGF- β production and secretion from human cells and platelets (1, 4). Recently, we reported that the levels of TGF- β are significantly increased in the sera of patients with EBV-associated, undifferentiated, and poorly differentiated nasopharyngeal carcinoma (NPC) (25). EBV is a ubiquitously occurring human gamma herpesvirus and has been etiologically associated with several disease conditions, including different malignancies and lymphoproliferative disorders (reviewed in reference 20). Primary infections, which usually occur in childhood, present with mild symptoms and are generally self-limiting. In industrialized Western countries, primary infections are often delayed until adolescence and are the major cause of glandular fever or infectious mononucleosis (IM). The infected individuals become lifelong virus carriers, and a proportion of these may develop chronic active EBV infection (CEI) with unusually high titers of antibody to a variety of EBV antigens. Thus, the

spectrum of EBV-induced diseases varies considerably: the infected individuals may remain healthy virus carriers, or they may develop a variety of disease conditions, likely depending on a number of factors, chief among these being the host's immune response. Furthermore, EBV is known to infect and immortalize human and simian B cells into continuously growing lymphoblastoid cell lines in vitro and to produce tumors in experimentally infected New World primates (20). In vivo, EBV genome is found in cells of endemic African Burkitt's lymphoma (BL), NPC, Hodgkin's disease, B-cell lymphomas, and oral hairy leukoplakia in AIDS patients. Given our interest in the parameters related to pathogenesis and the host's immune response in different EBV-associated diseases, in this study we sought to determine the levels of TGF- β in patients with these disease conditions.

Serum samples were obtained from healthy EBV-seropositive and -seronegative volunteers and from patients suffering from EBV-induced IM, CEI, BL, and NPC. The criteria for the diagnosis of these patients have been detailed in our recent publications (24, 25). The CEI patients in this study had prolonged severe relapsing courses of IM with high titers of anti-virus capsid antigen and EBV early antigen antibodies in serum and little or no antibody to the EBV nuclear antigens. Since TGF- β is secreted in a latent form (which becomes activated by a yet poorly understood mechanism before interacting with the TGF- β receptors on cells), we measured the

TABLE 1. Levels of TGF- β 1 in different groups of subjects

Patient condition	No. tested	Concn (ng/ml) ^a of TGF- β 1		Ratio (A/T) ^b
		Active	Total	
HI	20	6.69 $\pm$ 0.61	21.64 $\pm$ 0.76	0.31
IM	21	8.39 $\pm$ 0.54	24.33 $\pm$ 0.77	0.34
CEI	33	13.23 $\pm$ 0.65	32.52 $\pm$ 1.32	0.41
HD	14	8.22 $\pm$ 1.02	23.57 $\pm$ 1.98	0.35
BL	6	27.69 $\pm$ 1.99	57.12 $\pm$ 3.08	0.48
NPC	53	35.91 $\pm$ 2.29	64.86 $\pm$ 6.61	0.55

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^a Values are means $\pm$ standard errors.

^b A, active form; T, total.

TABLE 2. *P* values^a for differences in mean levels of TGF- β 1 between patients with different disease conditions

Patient condition	<i>P</i> value for comparison with:				
	IM	CEI	BL	NPC	HD
Active TGF- β 1					
HI	NS	<0.001	<0.001	<0.001	NS
IM		<0.001	<0.001	<0.001	NS
CEI			<0.001	<0.001	<0.001
BL				<0.05	<0.001
NPC					<0.001
Total TGF- β 1					
HI	NS	<0.001	<0.001	<0.001	NS
IM		<0.001	<0.001	<0.001	NS
CEI			<0.001	<0.001	<0.001
BL				NS	<0.001
NPC					<0.001

^a The unpaired Student *t* test was used to determine *P* values for differences between groups. NS, not significant.

concentrations of total TGF- β as well as of its active form in serum by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Promega, Madison, Wis.) as we described earlier (25). The mean values for different groups were compared by using Student's *t* test as described earlier (24). Table 1 depicts the average $\pm$ standard error of the levels of different forms of this cytokine in these disease conditions. Sera from NPC patients contained the highest concentration of this cytokine. This was followed by sera from patients with BL and CEI. The levels of this cytokine in serum did not differ significantly ($P > 0.05$) among healthy volunteers (including two EBV-seronegative individuals) and IM and Hodgkin's disease patients. The levels of total TGF- β and of active form were significantly elevated in NPC, BL, and CEI patients compared to other groups ($P \leq 0.01$) (Table 2).

Since TGF- β , in synergism with interleukin 10 (IL-10), can induce switching to immunoglobulin A (IgA) in antibody-producing B cells (23), and the EBV genome carries a human IL-10 gene homologue, i.e., the BCRF1 open reading frame (14), we determined whether high TGF- β levels in these EBV-associated disease conditions had any relationship with their anti-EBV antibody profiles. For this purpose, we chose to measure the EBV envelope glycoprotein 350 (gp350)-specific IgG, IgM, and IgA titers as well as antibody-dependent cellular cytotoxicity (ADCC)-mediating antibodies to gp350 in these

sera. As a unique tool for these determinations, we used our gp350-expressing, cloned human T cells, following our published protocols for indirect immunofluorescence and ADCC assays (24). gp350 is the major virion structural glycoprotein, against which most of the anti-EBV cellular and humoral immune responses are directed; anti-EBV subunit vaccines based upon this glycoprotein have afforded protection in experimental animals (monkeys) and have been the subject of clinical trials in humans (5, 15). As shown in Table 3, higher TGF- β levels correlated with increased positivity of sera for anti-gp350 antibodies of the IgA isotype. We further analyzed the relationship between TGF- β levels and different anti-gp350 titers by multiple regression analysis (25). As shown in Table 4, irrespective of the disease condition, TGF- β levels correlated positively ($P \leq 0.01$) only with gp350-specific IgA titers, suggesting that higher TGF- β levels in these patients may be driving (probably along with human IL-10 and/or its viral homologue) the enhanced production of anti-EBV IgA. On the other hand, a significant negative correlation ($P \leq 0.05$) was found with anti-gp350 IgM antibodies (Table 4). This finding is consistent with the documented decrease of virus-specific IgM in chronic viral infections. In this context, we have recently shown a positive correlation between the titers of gp350 IgA and virus capsid antigen-specific IgA in different EBV-associated diseases (24). Furthermore, a positive correlation between anti-gp350 IgA and anti-early antigen IgA titers has been reported (18). It is noteworthy that we found anti-gp350 IgA only in the sera of patients with EBV-associated diseases and not in any serum sample from healthy EBV-seropositive persons (i.e., 18 individuals) tested. In this regard, Yao et al. (26), using ELISA to determine anti-gp350 IgA, had found that 20 to 30% of sera from healthy EBV-seropositive subjects were positive for this antibody. Although the reasons for this difference in findings between these reports are not clear, it could result from the use of a different assay in each of these studies, suggesting that the indirect immunofluorescence assay (23) we used is more specific and/or less sensitive than the ELISA method used by Yao et al. (26).

TGF- β is an immunosuppressive cytokine; therefore, it is not surprising that many tumor cells secrete it to dampen antitumor immune responses. Interestingly, TGF- β has been suggested as a useful biomarker for certain tumors, e.g., breast, liver, and prostate cancers (6, 10, 21). We recently reported its high concentrations in the sera of NPC patients (25). Our present findings indicate that its levels are also increased in BL and CEI patients. Although further studies are needed to de-

TABLE 3. Correlation of levels of TGF- β 1 in serum with EBV gp350-specific antibodies

TGF- β 1 level (ng/ml)	No. tested	EBV gp350-specific antibody							
		IgG		IgM		IgA		ADCC	
		%	GMT ^a	%	GMT	%	GMT	%	GMT
Total									
≤ 29.99	51	92.16	89.31	50.98	14.94	7.84	1.25	62.75	28.23
30.00–39.99	19	89.47	158.41	42.11	6.16	42.11	3.36	84.21	88.59
40.00–49.99	18	66.67	39.29	27.78	2.45	50.00	3.59	44.44	7.17
≥ 50.00	36	83.33	71.69	41.67	2.78	61.11	6.27	77.78	73.54
Active									
≤ 14.99	62	90.32	93.84	46.77	11.26	11.29	1.45	67.74	38.06
15.00–29.99	35	74.29	54.23	40.00	4.17	51.43	3.98	51.43	15.95
≥ 30	27	88.89	78.07	40.74	2.78	66.67	5.36	88.89	90.98

^a GMT, geometric mean titer.

TABLE 4. Multiple regression analysis between levels of serum TGF- β 1 and EBV gp350-specific antibodies

Dependent variable	Independent variable	Coefficient	<i>t</i> test value	<i>P</i>
Active form of TGF- β 1	gp350-specific ADCC	-0.0013	0.3361	0.7374
	Anti-gp350 IgG	-0.0011	0.2915	0.7712
	Anti-gp350 IgM	-0.0182	3.1287	0.0022 ^a
	Anti-gp350 IgA	0.0302	2.1919	0.0303 ^a
Total TGF- β 1	gp350-specific ADCC	-0.0006	0.1015	0.9194
	Anti-gp350 IgG	-0.0007	0.1210	0.9039
	Anti-gp350 IgM	-0.0280	3.1556	0.0020 ^a
	Anti-gp350 IgA	0.0495	2.3494	0.0205 ^a

^a Significant ($P \leq 0.05$) correlation.

termine the exact source(s) of this enhanced TGF- β in these EBV-associated diseases, we speculate that its induction may result from enhanced EBV replication, alone or in association with its production from tumor cells. It is noteworthy, in this context, that we have recently shown that binding of EBV to its receptor, CR2 (complement receptor II, or CD21) on human platelets causes the release of TGF- β from the latter (1).

The increased TGF- β concentrations described here may play a pathogenic role in EBV-associated disease conditions. The cytokine is known to cause disruption of viral latency and stimulate EBV replication in Burkitt's lymphoma cells (18). Although TGF- β suppresses the growth of T, B, NK, and epithelial cells, EBV infection of susceptible cells renders them refractory to the growth-inhibitory effects of this cytokine (2). Certain cancers are known to develop mutations in either TGF- β receptors or their signaling pathway elements to avoid the tumor suppressor effects of TGF- β (7, 12, 27). As stated above, TGF- β is known to induce switching to IgA antibody production in B cells in combination with IL-10 (23). Consistent with these effects of TGF- β on antibody production is our finding of the positive correlation between higher levels of gp350-specific antibodies of the IgA isotype and the levels of TGF- β in EBV-associated disease conditions. Although IgA antibodies protect mucosal surfaces from invading pathogens (reviewed in reference 8), in the context of EBV infection they can potentially contribute towards pathogenesis; indeed, EBV-specific IgA is also known to mediate infection of epithelial cells which produce secretory component and transcytose IgA (22). This is relevant at least to the pathogenesis of NPC, as secretory-component-producing cells occur in the fossa of Rosenmuller, the anatomical location where NPC develops (16). This strongly suggests a role for IgA antibody in the development of NPC. Furthermore, anti-EBV serum IgA antibodies are known to have diagnostic and prognostic value in EBV-associated tumors and their elevated titers correlate with poor prognosis (reviewed in references 9 and 17).

Taken together, our present and previous studies suggest a role for TGF- β in the pathogenesis of NPC, BL, and CEI. This cytokine, therefore, may represent an appropriate molecular target for therapeutic intervention against these disease conditions and tumors as has also been suggested for other diseases (3).

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