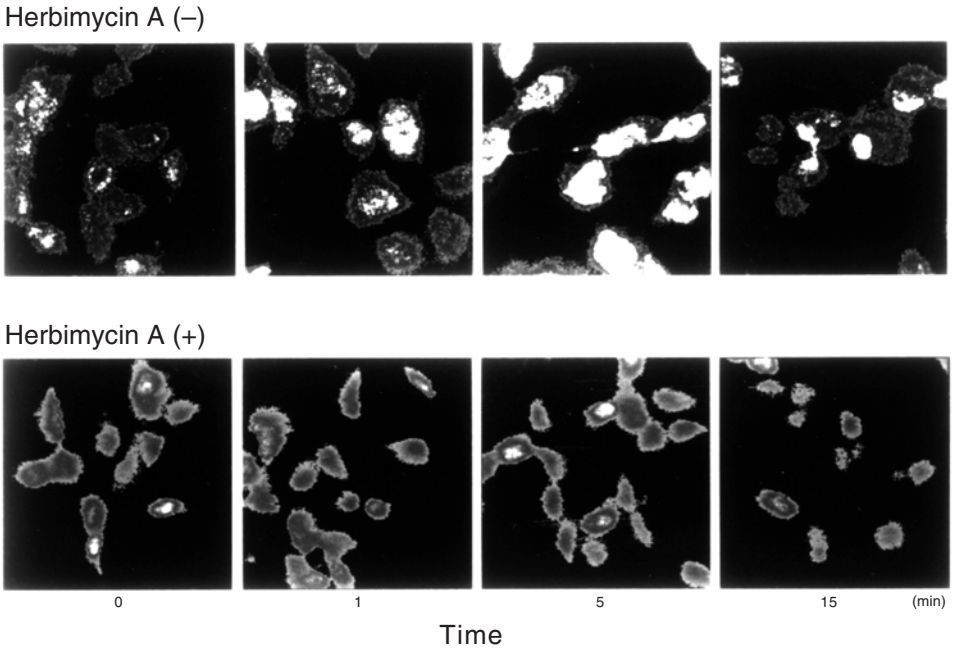

Fig. 3. Effects of herbimycin A on the interferon-gamma (IFN-γ)-induced phosphorylation of tyrosine. As shown in the upper panel, IFN-γ induced phosphorylation of tyrosine on HLC-1 cells. In the lower panel, pretreatment with herbimycin A appeared to inhibit the phosphorylation of tyrosine.

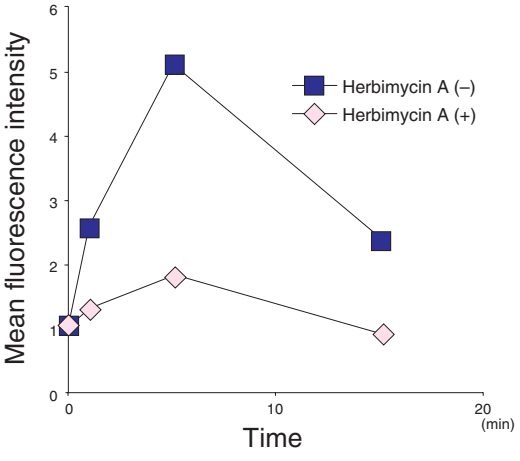

Fig. 4. Effects of herbimycin A on interferon-gamma (IFN-γ)-induced phosphorylation of tyrosine. Analysis of tyrosine phosphorylation by mean fluorescence intensity shows that IFN-γ increased the tyrosine phosphorylation five fold within 5 min. Herbimycin A inhibited the production of IFN-γ-inducible phosphotyrosine.

These findings indicate that IFN-γ induced tyrosine phosphorylation of cellular substrates which was inhibited by herbimycin A.

Discussion

Three intracellular signal transduction pathways have been considered in the induction of MHC antigens by IFN-γ in several types of cells (Koide et al., 1988; Klein et al., 1990; Nezu et al., 1990; Towata et al., 1991; Lahat et al., 1993); that is, the protein kinase A, Ca^{2+}-calmodulin and protein kinase C pathways. However, these pathways have not been shown to be decisive on the IFN-γ-induced expression of MHC class I and II antigens. There is only one report which states that tyrosine kinase pathways may be involved in the IFN-γ-induced expression of MHC class II (Ryu et al., 1993). Nevertheless, the detailed signal transduction mechanisms responsible for the IFN-γ-inducible expression of MHC antigens on cancer cells have not been elucidated, especially for MHC class I.

Recent biochemical studies have shown that IFN-γ activated the JAK1 and JAK2 tyrosine kinases which phosphorylate a down stream signal transducer and activator of transcription

(STAT) in the IFN-γ response signal pathway. Phosphorylated STAT1α dimerizes, translocates to the nucleus, and binds specific DNA elements, i.e., IFN-γ activation sites, thereby activating transcription factors, e.g., interferon regulatory factor 1 (Shuai et al., 1993a, 1993b; Silvennoinen et al., 1993; Darnell et al., 1994; Taniguchi et al., 1995).

We used an inhibitor of tyrosine protein kinase, herbimycin A (Uehara et al., 1989; Obinata et al., 1991), to determine whether tyrosine phosphorylation may be involved in the signal transduction for IFN-γ-induced expression of MHC class I and II molecules on HLC-1 cells. However, while herbimycin A is useful in determining whether tyrosine phosphorylation is involved in the mechanisms for activation by receptor-mediated signal transduction, it is not specific for JAK tyrosine kinases.

We observed that IFN-γ induced the expression of MHC class I and II on HLC-1 cells and the phosphorylation of protein tyrosine in their cellular substrates within 5 min. Herbimycin A inhibited both these expressions and the enhancement of tyrosine phosphorylation. The findings that IFN-γ activated the protein tyrosine kinases that phosphorylate the cellular substrates and induce MHC class I and II molecules suggest that tyrosine phosphorylation is involved in the induction of the expression of MHC molecules on HLC-1 cells by IFN-γ. Therefore, a JAK-STAT signal transduction pathway must be involved in the IFN-γ-induced expression of MHC class I and II molecules. However, we cannot exclude the possibility of involvement by other pathways because herbimycin A did not completely inhibit IFN-γ-inducible expression of MHC molecules. While the expression of MHC molecules is known to be regulated by other cytokines such as tumor necrosis factor alpha and interleukin 1 alpha (Seong et al., 1991; Sedlak et al., 1992; Wolchok and Vilcek, 1992), little is known about the signal transduction mechanisms which induce MHC antigens by these cytokines. Further, there are some reports which state that c-*myc* oncogene regulates the expression of MHC molecules on cancer cells (Versteeg et al., 1988; Gaforio et al., 1991). We think it should also be necessary in the future to clarify the signal transduction pathways by these cytokines and oncogenes for understanding the more detailed mechanisms which induce MHC molecules.

The expression of MHC class I and II antigens on cancer cells are involved in a variety of immune functions affecting tumor immunity. Cancer cells have reduced expression of MHC molecules enabling them to escape from the host's immunosurveillance system (Goodenow et al., 1985; Festenstein et al., 1986). Therefore, we think it is important to elucidate both the mechanisms modulating the expression of MHC molecules and the methods restoring the expression of these molecules on cancer cells for improvement of clinical problems about tumor immunity. In the present study, we elucidated the fact that tyrosine phosphorylation played an important role as the mechanism which restores the expression of MHC molecules on cancer cells.

In conclusion, tyrosine phosphorylation is considered to be an essential factor in the IFN-γ-induced expression of MHC class I and II molecules on HLC-1 cells.

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