INTRODUCTION

The interferons have been the first biomodifiers to become available in highly purified recombinant form (1). Of the three types of interferons, from leukocytes (IFN-α), fibroblasts (IFN-β), and immune T-lymphocytes (IFN-γ), IFN-α has been the first and most widely used in clinical studies.

Even though the nonconventional cytostatic and immuno-modulatory properties of IFN-α have widened its applicative spectrum well beyond the conventional field of antiviral therapy (reviewed in Ref. 2), its antineoplastic effects do not favorably compare with those of conventional cytostatic drugs, except in certain hematological malignancies (3). Some or all of a number of variables in clinical protocols may be involved in determining a poor clinical response in solid tumors. Among these variables, of major relevance appear to be the inappropriate dosage, schedule, and route of administration; the particular subtype of IFN-α utilized; the histiotype and stage of differentiation of the neoplastic disease; as well as the individual susceptibility of tumor patients (2, 4, 5).

At variance from conventional antiblastics, there are no preset, generally accepted rules to optimize treatment protocols with biomodifiers. One significant advance in this direction would be a consensus method to objectively evaluate the in vivo efficacy of IFN-α. Unfortunately, the levels of IFN-α in blood, although routinely assessed, do not appear to be suitable to this aim, since they have a short half-life (2, 4, 6) and do not provide any indication as to the biological activity of IFN-α on its cellular targets. A direct estimate of a molecular response, on the contrary, might represent a method of choice to measure interferon efficacy.

Among the gene products the expression of which is enhanced by IFN-α, class I MHC antigens have been long known for their elective susceptibility to in vitro up-regulation in a variety of cell types (7, 8) including PBMCs (8). Should this occur also in vivo, in humans, class I antigens might be useful to monitor the efficient delivery of pharmaceutical doses of IFN-α to different cell types and anatomical districts. Because PBMCs can be easily collected prior to and following interferon treatment, we have tested mRNA accumulation, synthesis, plasma membrane expression, total cellular steady state of class I MHC products in these cells, and their circulating levels in the plasma of nine malignant melanoma patients enrolled in a phase I trial involving treatment with escalation doses of recombinant IFN-α.

MATERIALS AND METHODS

Interferon, Melanoma Patients, and Experimental Plan. Recombinant IFN-α2 was from Hoffmann La Roche, Nutley, NJ. Patients with metastatic melanoma (stages III and IV of the M. D. Anderson Hospital classification) had received no previous chemotherapy except patient LNG, who had terminated a 4-(dimethyltriazeno)imidazole-4-carboxamide cycle 3 months before IFN-α administration. Nine melanoma patients were given i.m. injections of 3 × 10⁶ units of IFN-α three consecutive times at 24-h intervals. The treatment was repeated weekly for two more cycles at dosages of 9 × 10⁶ and 18 × 10⁶ units, respectively. Heparinized blood was collected prior to the first cycle and 24 h after the last injection of each of the three cycles and immediately tested, as described below, for class I MHC expression at plasma membrane, translational, or mRNA levels.

Because the IFN-α-mediated plasma membrane enhancement of class I molecules lasts longer than mRNA and translational increases (9–12), the present study was aimed to measure changes in the steady state cellular accumulation of class I protein products. The time interval between IFN-α administration and testing (24 h) was not preliminarily tested but was chosen in order to measure changes in the expression of class I antigens, keeping into account several parameters affecting antigenic modulation, such as: (a) the limited period (3–6 h) of pharmacologically active IFN-α levels in blood (2, 4, 5); (b) the time (up to 12 h) required by IFN-α to trigger the cellular processes resulting into enhancement of class I expression at protein level (11); and (c) the finite (about 24 h) half-life of antigenic modulation at cell surface after IFN-α withdrawal (9, 11). However, because many of the above referenced studies have been performed in continuous cell lines of different lineages, the conditions selected by us for PBMC testing may be suboptimal.

Reagents. MoAb W6/32 recognizes a class I determinant highly dependent on the association of the heavy (M, 12,000) chain with β₂-m (M, 12,000) (13). MoAb Namb-1 is to a distinct determinant carried by β₂-m (14). MoAb Ep2 is to a proteoglycan not expressed on PBMCs (15). MoAbs were purified from ascitic fluids by DEAE chromatography (Pharmacia, Uppsala, Sweden), following the instructions of the
obtained by routine Ficoll-Isopaque density gradient centrifugation of (12) and were used as controls. The cell lines WI-L2 and K562 have been centrifugation at 7000 × g for 5 min. Equalization of protein concentration in different extracts was confirmed by the BCA assay (Pierce, Rockford, IL). Both PBMCs and plasma were stored at −20°C in aliquots. The double determinant immunoassay to quantify soluble class I antigens was performed on polyvinyl chloride microtiter plates as described (18). Briefly, 10 μg of MoAb W6/32 to class I molecules (catcher) per well were absorbed on polyvinyl chloride plates; then the antigen source in duplicate (50 μl of undiluted extract or plasma) was added. After unbound antigen was washed, 125I-labeled MoAb Namb-1 (20 ng) was incubated (tracer). Following extensive washing, specifically bound MoAb Namb-1 was measured in a gamma-counter. Specificity controls included the use of irrelevant antibodies as catchers as well as tracers, and of soluble extracts from the class I-negative K562 cell line. Titration experiments were preliminarily performed to exclude testing in antigen excess. Indirect immunofluorescence was performed as described (19), with MoAbs at a concentration of 50 μg/ml on acetonefixed cytocentrifugates using 1 × 10⁶ cells as target. Freezing of fixed specimens at −20°C for up to 6 months did not alter MoAb W6/32 reactivity with PBMCs. Protein Synthesis and mRNA Analysis. Metabolic labeling of PBMCs with [35S]methionine and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of indirect immunoprecipitates were performed as described previously (10, 11). Total cellular RNA was obtained from guanidinium isothiocyanate lysis of PBMCs, run on a 2.2 M formaldehyde/1.4% agarose gel, transferred to nylon filters, and hybridized as described (11).

RESULTS

Effect of in Vivo IFN-α Treatment on the Levels of Class I MHC Molecules in the PBMCs and Plasma of Melanoma Patients. Nine melanoma patients underwent the three cycles of IFN-α administration as outlined in “Materials and Methods.” PBMCs and plasma samples were collected prior to IFN-α treatment and 24 h after the last administration of each cycle (Fig. 1). As expected, the class I molecular complex was detected in all extracts as well as plasma samples. The levels in 5 × 10⁶ PBMCs or 50 μl of plasma from untreated patients were found to be approximately equal to those present in soluble extracts from 2 × 10⁶ WI-L2 B-lymphoid cells and showed minimal variations among all patients. Levels of class I MHC antigens were recovered from the supernatant. These experiments (not shown) confirm that the increases in class I MHC expression are of similar magnitude in all mononuclear cell types. Representative results of this kind of analysis are shown in Fig. 2. In two additional cases, PBMCs were fractionated according to a procedure described in detail elsewhere (20). After recovery of adherent cells (1 h incubation in plastic flasks), positive selection of surface immunoglobulin cells was carried out by panning on anti-human immunoglobulin antibody-coated polystyrene dishes. Finally, adherence-negative/immunoglobulin-positive cells (T-enriched fraction) were recovered from the supernatant. These experiments (not shown) confirm that the increases in class I MHC expression are of similar magnitude in all mononuclear cell types. In addition, the negligible alterations in differential blood counts before and after IFN-α treatment (not shown) argue against the possibility that changes in the cellular composition of the mononuclear cell pool might account for inter- or intrapersonal differences in the levels of class I MHC expression (not shown).

Parehthetically, it should be noted that only patient DNG (good class I responder) showed a complete clinical response to IFN-α treatment. This patient was still alive with apparently no sign of the disease 3 years after treatment. All the remaining patients, including patient MST, showing good class I enhancement, had only minimal or no clinical response. Whether a
correlation exists between class I responsiveness and clinical outcome remains to be evaluated in a larger number of patients.

Effect of in Vivo IFN-α Treatment on the Synthesis and mRNA Accumulation of Class I MHC Products by the PBMCs of Melanoma Patients. In order to obtain information about the mechanisms involved in IFN-α up-regulation, the synthesis of class I protein products and the accumulation of specific mRNAs were assessed, in a distinct cycle of treatment, in two selected cases (BRR and MST, respectively). In patient BRR, PBMCs were isolated prior to (day 0) and following (day 4; 24 h after the last IFN-α administration) a cycle of three consecutive daily administrations of 18 × 10⁶ units of IFN-α; immediately processed in identical conditions by a metabolic pulse (2 h) in the presence of [35S]-methionine (50 μCi/ml), with no added IFN-α; and solubilized by the non-ionic detergent Renex 30. 32P Immunoprecipitation was done in parallel, with MoAb W6/32, from equal amounts of soluble extracts. As shown in Fig. 3, a residual IFN-α enhancement of class I heavy chain synthesis (slightly less than 2-fold) is still detectable at day 4 as compared to day 0 PBMCs, during the 2-h metabolic pulse. A similar quantitative increase of the associated β2-m component is also visible after longer exposure times (not shown). No increases in class I MHC synthesis could be demonstrated in the PBMCs from three additional, different melanoma patients by prolonging (8–24 h) metabolic labeling, most likely due to a rapid in vitro reversal of the IFN-α-mediated stimulation of class I synthesis (not shown). These results are consistent with the hypothesis that PBMCs exposed in vivo to IFN-α still display enhanced levels of translatable class I mRNAs 24 h after the latest interferon administration, and for at least the initial 24 h of in vitro permanence. To test this hypothesis directly, PBMCs were isolated as above at days 0 and 4 from patient MST and lysed immediately with guanidinium isothiocyanate solution. The isolated total cellular RNA was then tested by Northern analysis of 2-fold dilutions of untreated and IFN-α-treated PBMCs (same protocol as above), as indicated, followed by hybridization with the class I 32P-nick-translated third domain probe pS8. Controls included rehybridization of the same filter with a β-actin (β-act) probe and electrophoresis in parallel of K562 and WI-L2 RNAs, known to lack class I transcripts. The relative hybridization intensities of the 1.6-kilobase (Kb) pS8-generated and 1.7-kilobase β-actin bands in the absence (A) and presence (B), respectively, of IFN-α were estimated by densitometric scanning of preflashed, underexposed Kodak XAR-5 films. Densitometric values (arbitrary units) generated by the two probes in the same lane were plotted in graphic form with respect to each other. The lesser the amount of mRNA loaded, the closer the points are displayed to the origin of the axes. Under the assumption that hybridizable β-actin transcripts are not affected by IFN-α treatment, the slope of the lines is directly proportional to class I mRNA expression.

DISCUSSION

In this report we show that in vivo administration of IFN-α up-regulates the steady state protein levels and plasma membrane expression of class I MHC molecules in the PBMCs of
molecules. Although of lesser magnitude and limited life span, in the cellular source of class I molecules in plasma, their origin, at least in part, from PBMCs, is suggested by the related time sequence and magnitude of class I enhancement in most PBMCs and plasma samples.

Although the biochemistry and function of soluble class I antigens are, at present, poorly understood (26-28), one study performed with reagents and methods similar to ours (26) has recently shown that circulating class I molecules are comprised of both the classical HLA-A,B,C antigens and the nonconventional, alternatively spliced class I MHC products. Our data are equally compatible with IFN-α up-regulating either or both subsets. Whatever the nature of soluble class I molecules detected by us, their enhancement by IFN-α and their presence in the circulation in the form of a class I/β2-m complex confirm, in vivo, their release from nucleated cells in response to specific biological stimuli. These findings support the notion, still controversial (28, 29), that soluble class I antigens have a role in antigen-specific responses (27).

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REFERENCES


Class I Major Histocompatibility Complex Enhancement by Recombinant Leukocyte Interferon in the Peripheral Blood Mononuclear Cells and Plasma of Melanoma Patients

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