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Control of Tetrahydrobiopterin Synthesis in T Lymphocytes by Synergistic Action of Interferon-γ and Interleukin-2*

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Irmgard Ziegler‡, Karin Schott‡, Michael Lübbert§, Friedhelm Herrmann§, Udo Schwuléra¶, and Adelbert Bacher‖

From the ‡GSF-Institut für Experimentelle Hämatologie, München D-8000 70, the §Albert-Ludwigs-Universität, 1 Medizinische Klinik, Abteilung Hämatologie-Onkologie, Freiburg D-7800, ¶Biotest-Pharma, GmbH, Dreieich D-6072, and the ‖Lehrstuhl für Org. Chemie und Biochemie, Technische Universität München, Garching D-8046, Federal Republic of Germany

The control of (6*R*)-5,6,7,8-tetrahydrobiopterin (H_4biopterin) synthesis in primed T cells was analyzed by using the human T cell leukemia virus type I (HTLV-I)-transformed T cell line MT-2. In contrast to the slowly progressing induction of H_4biopterin synthesis during activation of resting T cells, it is completed during a 59-h period and is directed by a synergism of interferon-γ (IFN-γ) and interleukin-2 (IL-2). Both GTP cyclohydrolase and (6*R*)-(1′,2′-dioxopropyl)-5,6,7,8-tetrahydropterin synthase activities are induced by IFN-γ. They are further enhanced by combined treatment with IL-2, which *per se* is ineffective. Furthermore, the combined treatment synchronizes the time periods of both maximum activities, now extending from 33 to 44 h. This period correlates with high cellular H_4biopterin levels. It is preceded by a fast and transient period of H_4biopterin increase which depends on the synergistic action of both IFN-γ and IL-2. It coincides with a transient increase in sepiapterin reductase activity. In contrast to MT-2 cells, HTLV-I-transformed HUT 102 cells constitutively secrete IFN-γ and express IFN-γ mRNA. The accumulation of H_4biopterin is suppressed by anti-IFN-γ polyclonal antibody and correlates with constitutive expression of all H_4 biopterin-synthesizing enzymes.

It is well established that H_4biopterin[1] functions as the natural and immediate electron donor for hydroxylation of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (for review, see Ref. 1). According to this cofactor role for neurotransmitter biosynthesis and for phenylalanine degradation, high concentrations of H_4biopterin are found in competent tissues, such as adrenal medulla, hypothalamus, and liver (2). Recent evidence has shown that H_4biopterin is also synthesized during multiplication and differentiation of cells that lack neurotransmitter biosynthesis and phenylalanine degradation. This is the case during hematopoiesis and cellular immune response (for review, see Ref. 3). The accumulation of H_4biopterin proceeds gradually during lectin-induced blast transformation in murine (4) and human (5) T cells and is directed by consecutive increases in GTP cyclohydrolase and sepiapterin reductase activities (6). Increased GTP cyclohydrolase activities had also been observed after 72-h phytohemagglutinin treatment of resting human T cells (7).

IFN-γ was identified as the factor which causes increased neopterin synthesis and release in monocytes/macrophages (8). Recurrent periods of H_4biopterin synthesis in activated T cells were found to be induced by incubation with IFN-γ (5) as well as IL-2 (9). Although the induction of H_4biopterin synthesis during activation of resting T cells has been well documented, the operation of cytokines and the regulation of the biosynthetic enzymes in primed T cells have not been resolved so far. Moreover, the endogenous production of immunoregulatory lymphokines such as IFN-γ and IL-2 by activated human $CD4^+$ and $CD8^+$ T cells (10) may cause variable background levels.

T lymphocyte-derived H_4biopterin, in turn, was found to modulate IL-2 high affinity binding. Data suggest that this pterin participates in the control of IL-2 receptor assembly and is optimal at a concentration range of 10^{-8} to 10^{-7} M (11). The enhancement of IL-2-induced DNA synthesis which ultimately results from this feedback regulation of receptor affinity depends on a subtle inter-relationship of both lymphokine and H_4biopterin concentrations (12). This suggests the need for a stringent control of pterin biosynthesis, which has not been investigated so far.

In this study, we have used the HTLV-I-infected $CD4^+$ T cell lines HUT 102 and MT-2 to examine the control of H_4biopterin synthesis by IFN-γ and IL-2. In contrast to immune stimulation of T cells, HTLV-I infection leads to constitutive expression of both chains of the IL-2 high affinity receptor (for review, see Ref. 13). The expression of mRNA for IL-2 is, however, markedly lower in MT-2 cells as compared to HUT 102 cells. *In situ* hybridization with ^{35}S-labeled antisense RNA probes revealed 1–2% positive cells with 11–25 grains/cell, whereas only 0.3–0.8% positive cells with 11–25 grains were found in MT-2 cells (14). Our preliminary observation that MT-2 cells, in contrast to HUT 102 cells, produce neither IFN-γ nor H_4biopterin prompted us to use this cell line as a model system for activated human $CD4^+$ T cells and thus to examine IFN-γ and IL-2 as the two necessary signals which may regulate H_4biopterin synthesis.

It is generally accepted that during the *de novo* pathway of H_4biopterin synthesis, the formation of the first intermediate, dihydroneopterin triphosphate, from GTP is catalyzed by

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[1] The abbreviations and trivial names used are: H_4biopterin, (6*R*)-5,6,7,8-tetrahydrobiopterin; biopterin, 6-(L-erythro-1′,2′-dihydroxypropylpterin); H_2biopterin, 7,8-dihydrobiopterin; neopterin, 6-(D-erythro-1′,2′,3′-trihydroxypropylpterin); 6-pyruvoyl-H_4pterin, (6*R*)-(1′,2′-dioxopropyl)-5,6,7,8-tetrahydropterin; FCS, fetal calf serum; IFN-γ, interferon-γ; IL-2, interleukin-2; HPLC, high performance liquid chromatography; MOPS, 3-(*n*-morpholino)propanesulfonic acid; HTLV-I, human T cell leukemia virus type I.

GTP cyclohydrolase (EC 3.5.4.16) (Ref. 15; for review, see Ref. 16) and that IFN-γ increases the activity of this enzyme in monocytes/macrophages (7). More recent evidence suggests that the later steps of H_4biopterin synthesis proceed via tetrahydropterin intermediates (17–20). Upon triphosphate elimination from dihydroneopterin triphosphate, 6-pyruvoyl-H_4pterin synthase catalyzes an intramolecular reaction yielding the metastable intermediate 6-pyruvoyl-H_4pterin. The hydride equivalents for the reduction of this diketo compound are provided by NADPH. Sepiapterin reductase (EC 1.1.1.153) potentially catalyzes the reduction of each of the 1′- and 2′-oxo functions (21) to yield the final product, H_4biopterin. 6-Pyruvoyl-H_4pterin reductase, which has been proposed to be responsible for specific reduction of the 2′-keto group (22, 23), was isolated (24) and was shown to be identical to aldose reductase (EC 1.1.1.21) (25). It appears not to be required in all tissues (26). Measurement of the specific contribution of this enzyme to H_4biopterin synthesis in crude cell extracts is not possible to date. In this study, we therefore investigated the individual and specific control of GTP cyclohydrolase, 6-pyruvoyl-H_4pterin synthase, and sepiapterin reductase activities by synergism of IFN-γ and IL-2.

EXPERIMENTAL PROCEDURES

Materials—The following materials were obtained from the manufacturers in parentheses: RPMI 1640 medium and FCS (GIBCO); L-glutamine, penicillin, and streptomycin (Biochrom, Berlin); human recombinant IFN-γ (Boehringer Mannheim); nIFN-γ (from phorbol ester-stimulated human peripheral blood mononuclear cells, >99% pure) (Biotest, Frankfurt); human recombinant IL-2 (Boehringer Mannheim and Cetus Corp.); natural IL-2 (Lymphocult® T-HP, Biotest); IFN-γ radioimmunoassay test kit (Lentocor, Melvern, PA); human IFN-γ rabbit polyclonal antibody (Paesel, Frankfurt); guanidinium isothiocyanate and Kodak X-AR5 x-ray film (Eastman Kodak); Nytran membrane (Schleicher & Schüll); and [α-^{32}P]dCTP (Amersham Corp.). The sources of the chemicals which were used for biopterin determination (4), for determination of enzymatic activities (27, 28), and for protein determination (27, 28) were as previously described.

Culture and Harvesting of Cells—The HTLV-I-infected T cell lines MT-2 and HUT 102 (ATCC TIB 162) were donated by Dr. van der Helm (Max-von-Pettenkofer-Institut, München, Federal Republic of Germany) and by Dr. I. Minowada (Roswell Park Memorial Institute, Buffalo, NY), respectively. Originally, the MT-2 cell line was established by Dr. I. Miyoshi (Kochi Medical School, Kochi, Japan) from umbilical cord lymphocytes that had been transformed by HTLV-I following co-cultivation with leukemia cells from patients with adult T cell leukemia lymphoma (29), whereas the HUT 102 cell line was derived from peripheral blood of a patient with cutaneous T cell lymphoma (30). Both cell lines were propagated twice and then frozen. Aliquots were used for the experiments until they had reached 12–14 passages. Two starter cultures of MT-2 cells were obtained whose number of previous passages was unknown. HL-60 cells (ATCC CCL 240) was from Dr. H. P. Koeffler (UCLA, Los Angeles). The cells were maintained in RPMI 1640 medium supplemented with L-glutamine (2 mM), $NaHCO_3$ (0.2%), penicillin (100 units ml^{-1}), and streptomycin (100 μg ml^{-1}). 10% heat-inactivated FCS was added. The cells were seeded at a density of 1×10^5 cells ml^{-1} and cultured at 37 °C in humidified air containing 5% CO_2. IFN-γ and IL-2 were added from the stock solutions which were reconstituted from lyophilized preparations according to the manufacturer's instructions or were aliquoted from the commercially obtained solutions in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline/bovine serum albumin (1%). There was no apparent difference in the cellular response regardless of whether natural or recombinant IL-2 and IFN-γ were used. At the periods indicated, viable cells were counted by trypan blue exclusion, centrifuged at 400 × *g* for 10 min, and stored at −70 °C.

HPLC Determination of Biopterin—Cellular H_4biopterin was determined in aliquots of 5×10^6 cells after extraction in 1 ml of 0.1 M HCl and iodine oxidation to biopterin. For quantification of H_2biopterin, which, in contrast to H_4biopterin, can be recovered as biopterin after oxidation also in alkaline solution (2), the oxidation step was performed in 0.1 M NaOH, followed by addition of 2 N HCl to bring biopterin back to its cationic form. Deproteinization by trichloroacetic acid, prepurification by cation-exchange chromatography, separation by HPLC, and fluorometric detection have been described previously (4). Modifications of the method were the same as detailed in Ref. 27. Biopterin concentrations <2 pmol/10^6 cells have to be attributed to its uptake from FCS, which contains variable amounts of this pterin (4). The values for cellular biopterins include the trace amounts due to presumable uptake from the medium. For analysis of the cell culture supernatant, 1-ml aliquots were deproteinized with 0.5 ml of trichloroacetic acid (2 M) and were subsequently processed in the same way as for the determination of cellular biopterin. The biopterin from FCS was subtracted as background value. Each measurement was carried out in duplicate. For the purpose of statistics, *n* denotes the number of independent experiments.

Preparation of Cell Extracts and Enzyme Assays—Aliquots of 5×10^7 cells were extracted in a tapered tissue grinder (Wheaton, Millville, NJ) using a Teflon pestle with 700 μl of phosphate buffer (50 mM, pH 7.5) by mechanical stirring (500 revolutions min^{-1}) for 1 min. After centrifugation at 12,000 × *g* for 5 min, the supernatant was desalted on NAP-5 Sephadex G-25 columns as described before

TABLE I

Production of IFN-γ and reduced biopterins (H_4 biopterin and H_2 biopterin) by HUT 102 and MT-2 cells

IFN-γ was determined by radioimmunoassay in the culture supernatants of cells grown to a density of 7–9 × 10^5 cells/ml^{-1}. Biopterin was determined by HPLC after acidic oxidation (H_4 biopterin) and alkaline oxidation (H_2 biopterin), as described under "Experimental Procedures," of cell extracts and culture supernatants after harvest of cultures grown to 7–9 × 10^5 cells/ml^{-1}. Values are means ± S.D. ($n = 6$).

	HUT 102	MT-2
IFN-γ in culture supernatant (units/ml^{-1})	20.0 ± 2.0	1.0
H_4biopterin in cells (pmol/10^6 cells)	9.1 ± 2.1	<2.0[a]
H_2biopterin in cells (pmol/10^6 cells)	ND[b]	ND
Biopterins in supernatant	ND	ND

[a] Total of biopterin found after acidic or alkaline oxidation.
[b] ND, not detectable.

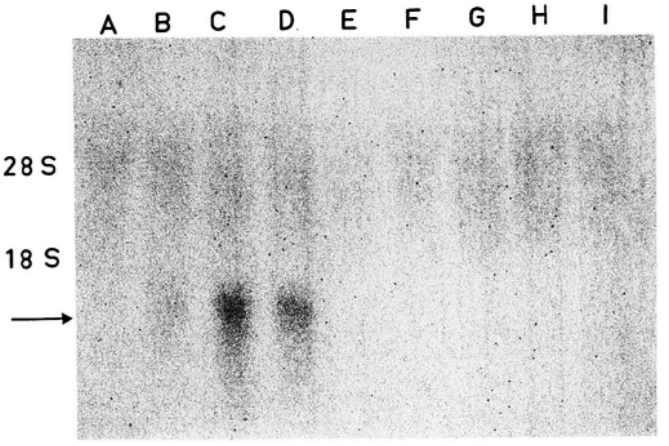

FIG. 1. **Northern blot analysis of IFN-γ mRNA expression in HUT 102 and MT-2 cells.** RNA was prepared from the cells, electrophoresed (15 μg/lane), and hybridized to an IFN-γ cDNA probe as described under "Experimental Procedures." The concentrations of IL-2 refer to units $milliliter^{-1}$ to which the cells were exposed for the periods indicated. RNA was prepared from: HL-60 cells as negative control (*lane A*), HUT 102 cells (*lane B*), HUT 102 cells plus 25 units of IL-2 for 12 h (*lane C*), HUT 102 cells plus 100 units of IL-2 for 12 h (*lane D*), MT-2 cells (*lane E*), MT-2 cells plus 25 units of IL-2 for 12 h (*lane F*), MT-2 cells plus 100 units of IL-2 for 12 h (*lane G*), MT-2 cells plus 25 units of IL-2 for 36 h (*lane H*), and MT-2 cells plus 100 units of IL-2 for 36 h (*lane I*). The 28 S and 18 S ribosomal RNAs served as molecular weight markers and are indicated. The *arrow* indicates IFN-γ mRNA.

(27). The activity of GTP cyclohydrolase was determined after iodine oxidation of the reaction products to neopterin phosphates, which were separated by ion-pair reverse-phase HPLC. The activity of sepiapterin reductase was determined after iodine oxidation of the reaction product to biopterin by reversed-phase HPLC. The detailed assay conditions for both enzymes are described in Ref. 27. 6-Pyruvoyl-H_4pterin synthase activity was determined by release of tritiated water from [2′-^{3}H]H_2neopterin triphosphate. The preparation of the tritium-labeled substrate from [4′-^{3}H]GTP (28) and the assay conditions were previously outlined (27, 28). Protein concentrations were determined as described (27). Each determination was done in duplicate. For statistical treatment, n denotes the number of independent induction experiments.

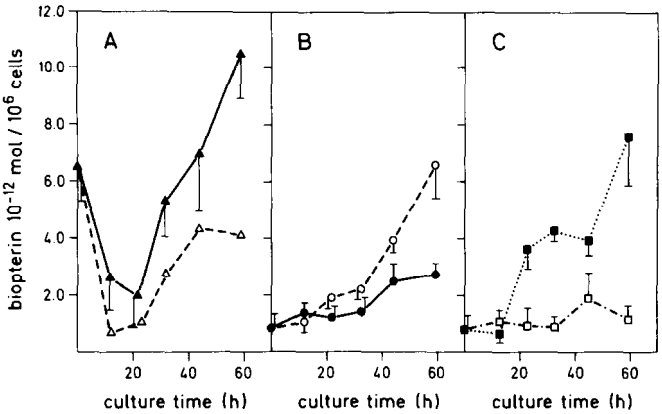

FIG. 2. **Time course of H_4biopterin production in HUT 102 and MT-2 cells.** Cells were harvested from a culture grown to a density of 7–9 × 10^5 cells ml^{-1} and reseeded at a density of 1 × 10^5 cells ml^{-1}. *A*, HUT 102 control cells (▲) with anti-IFN-γ antibody (neutralizing 1000 units ml^{-1}) (△); *B*, MT-2 control cells (●) with IFN-γ (1000 units ml^{-1}) (○); *C*, MT-2 cells with IL-2 (20 units ml^{-1}) (□) with IFN-γ (1000 units ml^{-1}) plus IL-2 (20 units ml^{-1}) (■). Values represent means ± S.D. (n = 4–6). The data for anti-IFN-γ antibody-treated cells represent the mean of two experiments which differed <10%.

TABLE II

Activities of H_4biopterin-synthesizing enzymes in cells grown to a density of 7–9 × 10^5 ml^{-1}

Values are means ± S.D. (n = 4 (GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase) and n = 6 (sepiapterin reductase)).

Cells	GTP cyclohydrolase	6-Pyruvoyl-H_4pterin synthase	Sepiapterin reductase
		pmol·mg^{-1}·min^{-1}	
HUT 102	0.214 ± 0.077	0.130 ± 0.023	327.8 ± 49.4
MT-2	0.055 ± 0.023[a]	0.077 ± 0.003	216.3 ± 47.0[a]

[a] In the cells derived from the second starter culture, the activity of GTP cyclohydrolase was 0.014 ± 0.003 (n = 6) and of sepiapterin reductase, 88.0 ± 30.0 (n = 4). The degree of increase after cytokine treatment, however, was the same. Therefore, the data of both starter cultures were pooled in Fig. 3 (*A* and *C*).

Interferon-γ Assay—The radioimmunoassay was performed according to the manufacturer's instructions with a 200-μl culture supernatant in triplicate. Units refer to standard calibration of the values provided with the test kit. They were obtained by the manufacturer by calibration using the National Institutes of Health standard. The minimum detectable dose is 0.1 unit ml^{-1}. S.D. refers to independent experiments.

Northern Blot for IFN-γ mRNA Expression—Total RNA was extracted from 5 × 10^7 cells after lysis in guanidinium isothiocyanate using the method of Chirgwin *et al.* (31). RNA was dissolved to a final concentration of 2–4 μg μl^{-1} in 10 mM Tris (pH 7.4) containing 1 mM EDTA and 0.1% sodium dodecyl sulfate and size-fractionated on a denaturing formaldehyde/MOPS gel (32). 28 S and 18 S ribosomal RNAs served as molecular weight markers and as indicators for integrity and equal loading of RNA. RNA was transferred onto nylon-based membrane with 10 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate (pH 7.0)) and fixed to the membrane by baking for 2 h at 80 °C *in vacuo*. The membrane was prehybridized and hybridized under conditions previously described (33). A full-length IFN-γ cDNA (1.2-kilobase *Sac*I/*Bam*HI restriction fragment) (34) was radiolabeled to high specific activity with [α-^{32}P]dCTP by random priming (35), and 3 × 10^6 cpm of freshly denatured radiolabeled probe were added to 3 ml of the hybridization mixture. Hybridization took place at 42 °C for 16 h, and the membrane was rinsed at 65 °C in decreasing concentrations of SSC to a final concentration of 0.1 × SSC for 15 min. Autoradiography was performed for 5 days at −70 °C with Kodak X-AR5 film with an intensifying screen.

RESULTS

Production of IFN-γ and Expression of IFN-γ mRNA—HUT 102 cells produce IFN-γ and secrete it into the culture medium, whereas no IFN-γ is detectable in supernatants of MT-2 cells (Table I). To examine further the difference between the cell lines, steady-state levels of IFN-γ mRNA were determined (Fig. 1). Accumulation of IFN-γ mRNA by HUT 102 cells correlates with the constitutive production of the protein. The absence of IFN-γ mRNA in MT-2 cells agrees with the lack of production of this protein. Since IL-2 is known to be required for maximum synthesis of IFN-γ (36), its effect on IFN-γ mRNA expression in both cell lines was examined. As seen in Fig. 1, IL-2 causes further increases in the levels of IFN-γ steady-state mRNA in HUT 102 cells. Such an induction was not achieved by IL-2 in MT-2 cells. Thus, in contrast to HUT 102 cells, MT-2 cells lack both constitutive and inducible IFN-γ mRNA expression.

Production of Reduced Biopterins (H_2biopterin and H_4biopterin)—Biopterin accumulates in HUT 102 cells and is present in the cells in its fully reduced form, H_4biopterin (Table I). In the culture supernatant, biopterin concentrations do not exceed the background levels which are caused by FCS in the medium (4). Fig. 2*A* shows the time course of

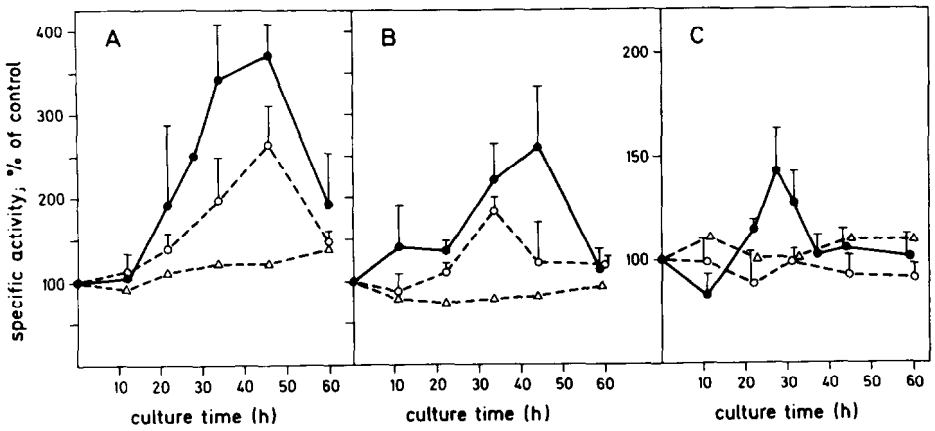

FIG. 3. **Induction of H_4biopterin-synthesizing enzymes in MT-2 cells by IFN-γ, IL-2, and combination of IFN-γ and IL-2.** *A*, GTP cyclohydrolase; *B*, 6-pyruvoyl-H_4pterin synthase; *C*, sepiapterin reductase. △, IL-2 (20 units ml^{-1}); ○, IFN-γ (500–1000 units ml^{-1}); ●, IFN-γ (500–1000 units ml^{-1}) plus IL-2 (20 units ml^{-1}). Points are means ± S.D. (n = 7 for GTP cyclohydrolase and sepiapterin reductase (except GTP cyclohydrolase at t_{27}, which is the mean of a duplicate determination)) and n = 6 for 6-pyruvoyl-H_4pterin synthase). The data for IFN-γ treatment with 500 and 1000 units ml^{-1} were not statistically different and were therefore pooled.

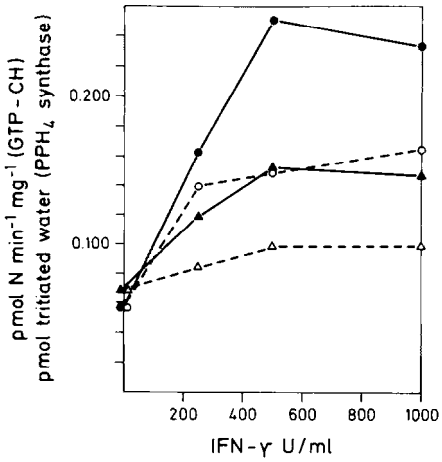

FIG. 4. **Induction of GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase in MT-2 cells by IFN-γ after 44 h and enhancement by IL-2.** ○ and ●, GTP cyclohydrolase (*GTP-CH*); △ and ▲, 6-pyruvoyl-H_4pterin (*PPH_4*) synthase. *Open symbols* and *dashed lines*, IFN-γ; *closed symbols* and *solid lines*, IFN-γ plus IL-2 (20 units ml^{-1}).

H_4biopterin synthesis in HUT 102 cells. Upon transfer of the cells from a densely grown culture to fresh medium, H_4biopterin levels show an initial decrease which levels off during the first 22 h. During the following period, renewed synthesis causes H_4biopterin concentrations to rebound to ~5-fold levels as compared to t_{22}. Values at t_0 are derived from cells which were resuspended in fresh medium and harvested after 5–15 min. Therefore, they are lower than those presented in Table I, which were obtained from cells immediately after harvesting from cultures grown to high density. Neutralization of constitutively produced IFN-γ with a polyclonal antibody against IFN-γ suppresses H_4biopterin production by HUT 102 cells (Fig. 2*A*). After 59 h, these cells regain only 60% of the biopterin levels found at $t = 0$. This corresponds to ~37% as compared to controls at $t = 59$ h. This indicates that IFN-γ is needed for induction of H_4biopterin synthesis. In contrast to HUT 102 cells, MT-2 cells show only trace amounts of biopterin, and no biopterin above culture medium level is found in the supernatant (Table I). After reseeding of the cells in fresh medium, the cellular levels do not exceed background values (Fig. 2*B*). H_4biopterin synthesis can be induced by IFN-γ, and the cellular concentrations average 6.5-fold amounts after 59 h. Higher values up to 10-fold levels have been obtained in some experiments. IL-2 proved to be incapable of inducing H_4biopterin synthesis (Fig. 2*C*). Combined treatment of the cells with IFN-γ plus IL-2, however, markedly enhances H_4biopterin production. This is especially true for the initial period of 22–27 h after seeding.

Activities of H_4biopterin-Synthesizing Enzymes—H_4biopterin accumulation in HUT 102 cells and its absence in MT-2 cells correlate with the activities of H_4biopterin-synthesizing enzymes in both cell lines (Table II). MT-2 cells are largely deficient in GTP cyclohydrolase activity. Neopterin production is around the detection limits (0.01–0.05 pmol min^{-1} mg^{-1}). The constitutive expression of 6-pyruvoyl-H_4pterin synthase activity, which in HUT 102 cells amounts to ~60% as compared to GTP cyclohydrolase, is further reduced in MT-2 cells. It approaches low levels similar to GTP cyclohydrolase. Sepiapterin reductase activity is only marginally lower than that in HUT 102 cells.

IFN-γ treatment of MT-2 cells induces an increase in GTP cyclohydrolase activity which starts after 11 h and reaches maximum levels after 44 h (Fig. 3*A*). IL-2 alone does not induce enzyme activity. Combined treatment of the cells with IFN-γ and IL-2 further enhances GTP cyclohydrolase activity. Due to the acceleration of the increase in enzyme activity, the proportion of enhancement was highest at ~33 h. Similarly as with GTP cyclohydrolase, IL-2 has no effect on 6-pyruvoyl-H_4pterin synthase activities. They are, however, analogously increased by IFN-γ treatment (Fig. 3*B*) and culminate after 33 h. As with GTP cyclohydrolase, combined treatment with IFN-γ and IL-2 further enhances the activity of 6-pyruvoyl-H_4pterin synthase. It delays decrease in enzyme activity so that the degree of induction is highest at 44 h. Therefore, in the cells subjected to this treatment, maximum activities of both GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase extend from 33 to 44 h.

Sepiapterin reductase activity is not changed by either IL-2 or by IFN-γ alone (Fig. 3*C*). Only the combination of both cytokines increases its activity to a smaller extent than is found for the two other enzymes. Maximum activity occurs at 27 h. Thus, sepiapterin reductase differs from both GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase with respect to signalling, extent of induction, and timing.

To characterize further the synergism of IFN-γ and IL-2, the induction of GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase by IL-2 was determined at different IFN-γ concentrations. As seen in Fig. 4, both enzyme activities approach plateau levels when IFN-γ concentrations exceed 200 units ml^{-1}. The additional induction achieved by combination with IL-2 does not level off at this saturating IFN-γ concentration, but rather continues to increase to IFN-γ concentrations of 500 units ml^{-1}. Thus, the degree of induction increases to this limit dependent on the concentration of IFN-γ.

DISCUSSION

In this study, we found that H_4biopterin synthesis in the HTLV-I-transformed T cell lines MT-2 and HUT 102 is directed by the synergistic action of IFN-γ and IL-2. This may explain the earlier observations that biopterin synthesis in activated human T cells which produce both synergistically acting cytokines (10) increases after addition of exogenous IFN-γ (5) or IL-2 (9). Furthermore, immunotherapy with interferons (37) and with IL-2 (3, 38, 39) enhances neopterin and biopterin levels in urine, serum, and lymphokine-activated killer cells. Our preliminary observation that HUT 102 cells, in contrast to MT-2 cells, produce both IFN-γ and H_4biopterin and the report (14) that HUT 102 cells show a markedly higher expression of IL-2 mRNA than MT-2 cells provided the rationale to select MT-2 cells as a model system for coherent analysis of the H_4biopterin-synthesizing system in activated T cells and its control by both cytokines. In contrast to the slowly progressing induction of H_4biopterin synthesis during activation of resting T cells (5–7), its regulation in primed T cells is largely unknown so far. We have shown that the activities of each of the enzymes involved in H_4biopterin synthesis (GTP cyclohydrolase, 6-pyruvoyl-H_4pterin synthase, and sepiapterin reductase) are subject to individual and specific regulation.

The first point to emerge was that in MT-2 cells, both constitutive GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase activities have similar values. The rate-limiting effect not only of GTP cyclohydrolase but also of 6-pyruvoyl-H_4pterin synthase has been reported for various tissues in man and monkey (40). This is in line with our findings in human T cells. However, GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase are coinduced in activated T cells by IFN-γ, a phenomenon which has not been described previously. IL-2 *per se* is ineffective, but causes further up-regulation of both enzyme activities in combination with IFN-γ. Moreover,

by acceleration of the increase in GTP cyclohydrolase activity together with a delay in the decrease of 6-pyruvoyl-H_4pterin synthase activity, the time periods of their maximum enzyme activity levels are coordinated. This results in a more extended interval of joint maximum activities.

In addition, our results demonstrate that sepiapterin reductase is subject to another type of control. Its activities are constitutively higher than those of GTP cyclohydrolase under conditions of substrate saturation and are not changed by IFN-γ or IL-2. Increase in its activity depends on the synergism of both cytokines, and the degree of induction is smaller than for the other two enzymes. Maximum levels are found ~27 h after seeding, and they clearly precede the time period for the maximum activities of the other two enzymes.

Furthermore, the data show that a heterogenous time course of cellular H_4biopterin production occurs after combined treatment with IFN-γ and IL-2. Maximum levels at time periods exceeding 33 h are satisfactorily explained on the basis of the well-established pathway of H_4biopterin synthesis (for review, see Ref. 16). At this time period, GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase operate with maximum activities. Constitutive sepiapterin reductase activity by far exceeds the activities of the other two enzymes, and H_4biopterin accumulation may thus not depend on further activation of this enzyme. An initial rapid accumulation of H_4biopterin results in an intermediate plateau of ~50% of the maximum. During this period from 22 to 27 h, however, the activity of 6-pyruvoyl-H_4pterin synthase almost remains at the starting level. This discrepancy suggests that additional mechanisms may contribute to the rapid initial accumulation of H_4biopterin. An immediate cessation of H_4biopterin degradation and/or export may explain this intermediate plateau in spite of continuing low levels of GTP cyclohydrolase and 6-pyruvoyl-H_4pterin synthase activities. Another hypothesis is the existence of a modified pathway of H_4biopterin synthesis which depends on combined treatment with IFN-γ and IL-2 and which correlates with a transient increase in sepiapterin reductase activities. It is tempting to speculate that this alternate route proceeds via the "salvage pathway." It has been postulated for a number of cell lines, and it has been suggested that side chain reduction by sepiapterin reductase and reduction of a 7,8-dihydropterin core by dihydrofolate reductase may be involved (41). The intermediate(s) remained unidentified, and the mechanisms await clarification. It is also not clear whether the transient increase in sepiapterin reductase activities during the initial period of H_4biopterin accumulation is physiologically relevant.

It has been suggested (7) that the IFN-γ-induced increase in GTP cyclohydrolase activity in monocytes/macrophages is due to activation by increased cellular GTP concentrations. Increased cellular substrate concentrations cannot explain the results reported above, which were obtained under conditions of substrate saturation. Whether the apparent modulation of enzyme activities by IFN-γ and by its synergism with IL-2 can be explained at the level of transcription and translation or by post-translational events such as enzyme phosphorylation awaits further investigation.

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I Ziegler, K Schott, M Lübbert, F Herrmann, U Schwuléra and A Bacher

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