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TUBULORETICULAR REORGANIZATION OF CYTOMEMBRANES IN CELLS TREATED WITH WITH HUMAN ALPHA INTERFERONS - A REVIEW

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Abstract

Human alpha interferons (IFN-a) cause a reorganization of internal cell membranes into tubuloreticular inclusions (TRI). Morphogenesis and cytochemistry indicate a pre-Golgi intracisternal origin from the endoplasmic reticulum. Clinically, TRI formation in human blood mononuclear cells correlates with systemic IFN-a treatment or with endogenous overproduction of IFN-a in viral or autoimmune diseases (e.g., rubella syndrome, AIDS, systemic lupus erythematosus). In vitro, TRI formation can be produced by treatment of Daudi lymphoblasts or vascular endothelial cells with IFN-a, and is blocked by actinomycin-D. In Daudi lymphoblasts or vascular endothelial cell cultures, TRI formation parallels induction of 2'-5' A synthetase, inhibition of thymidine kinase and growth inhibition; however, heavy water treatment of Daudi cells prevented TRI formation while induction of 2'-5' A synthetase and growth inhibition persisted. TRI formation was dissociated from IFN-a antiproliferative activity in a mutant clone of Daudi lymphoblasts. Decreased glycoprotein biosynthesis and increased phospholipid biosynthesis may accompany progressive TRI accumulation.

Key words: Alpha interferon, endoplasmic reticulum, tubuloreticular inclusions, electron microscopy, endothelium, lymphocytes, cytoskeleton, lipid chromatography, enzyme cytochemistry, non-human primates.

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Introduction

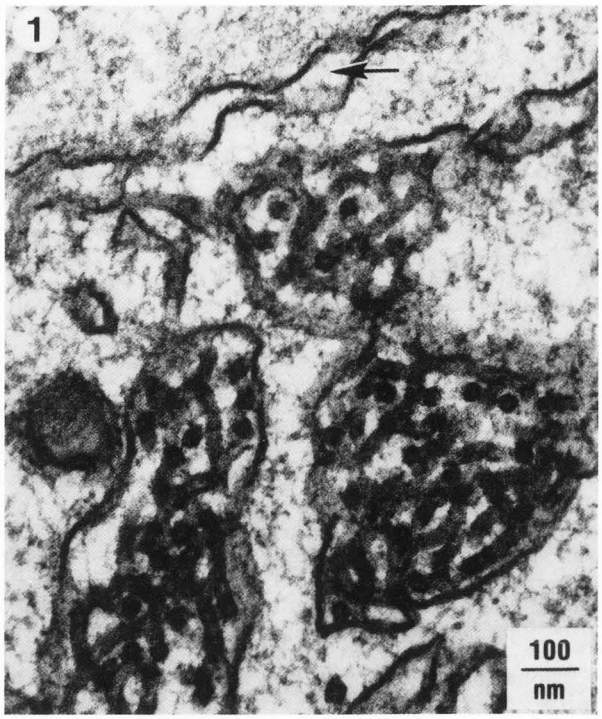
Ultrastructure of TRI

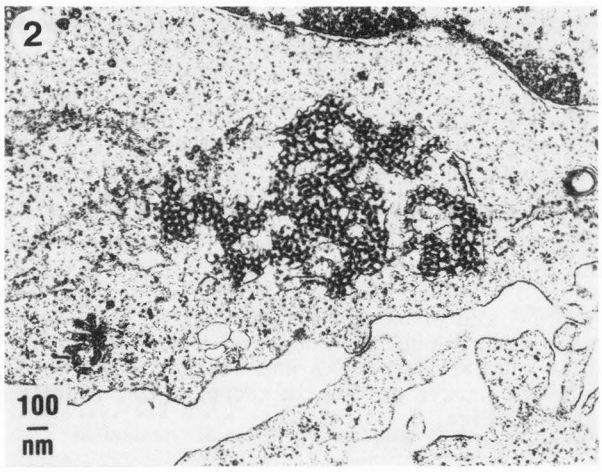
Incubation of lymphocytes, monocytes, or vascular endothelial cells with human type I interferons (alpha or beta) induces a characteristic change in the ultrastructure of pre-Golgi cytomembranes in human[12,17,21,26,46,47], ape[78] and monkey cells[11]. The rough endoplasmic reticulum (ER) and perinuclear envelope appear to be primary targets of this interferon effect[21], but annulate lamellae and the transitional complex of ER-Golgi membranes can also be sites of TRI formation[21,26,46]. The essential feature is accumulation of intracisternal membranous tubules which range from 22-28 nm diameter (Figs. 1, 2). These generally have been referred to as "tubuloreticular" inclusions or "TRI"[22].

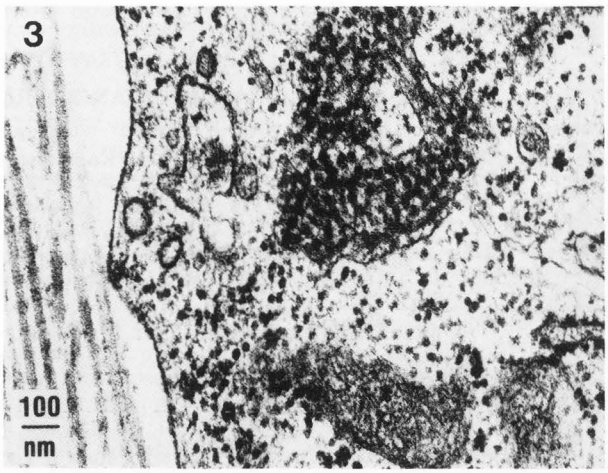
In non-human primate cells exposed to human alpha interferon (IFN-a), the membranous tubular elements often form compact, paracrystalline arrays within expanded cisternae of the ER[11,78] (Fig. 2). Even before the causation of TRI by IFN-a was established, it had been proposed that the paracrystalline membranous arrays in animal cells represented a functional variation of the loosely configured TRI found in human cells[63,79]. This hypothesis has been confirmed in recent studies of rhesus monkey placental cells[11] and of baboon lymphoblasts (Feldman, D, Grimley, PM, unpublished observations): both the irregular tubular meshworks and the geometric paracrystalline arrays develop in response to human IFa treatment (Figs. 3, 4).

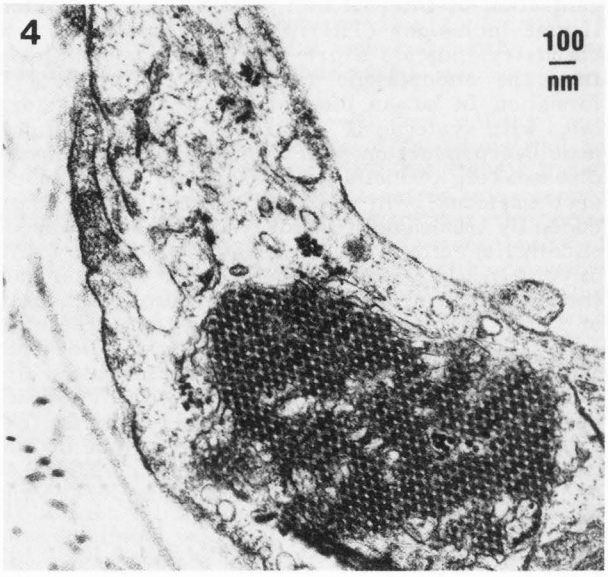
Morphogenesis of TRI

In human cells, TRI appear to originate as multiple, branching invaginations of the membrane bilayers bounding the ER or perinuclear cisternae (Fig. 1). The earliest tubule formation begins in the perinuclear region[21]. In Daudi lymphoblasts very loose networks of intracisternal membranous tubules develop within 12 to 24 hours after exposure to IFN-a in vitro[21]. These networks gradually become larger and more compact during prolonged IFN-a stimulation, but the tubular branching remains irregular. Blood lymphocytes develop TRI within 24 hours after IFN-a treatment in vitro or in vivo. These are usually smaller and more compact than the accumulations observed in lymphoblasts[17] but occasional "loose forms" are also identified. Regions of TRI developing in vascular endothelial cell cultures also are very small at initial observation; they tend to enlarge over a period of days[12]. In monkey cells, even the smallest regions of TRI typically exhibit a geometric









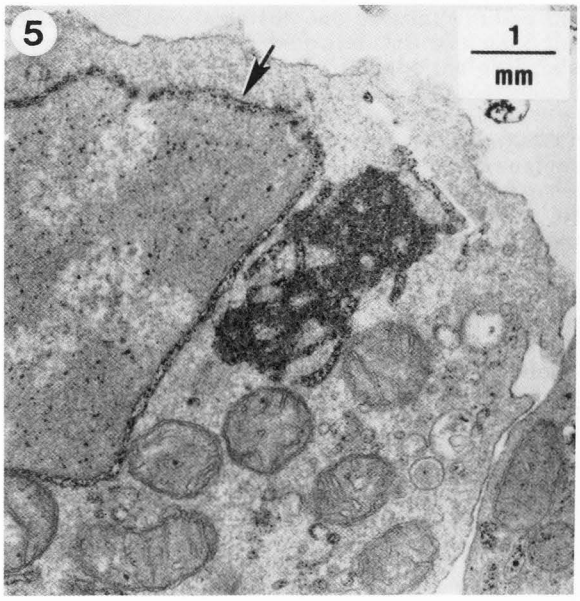


Fig. 1. Early TRI formation : intracisternal 25 nm membranous tubules in Daudi lymphoblast, 12 hours after treatment with IFN-a (500 IU/ml). Note proximity to perinuclear cisternum (arrow).
Fig. 2. Typical network of TRI in a human lymphoblast after treatment with IFN-a (500 IU/ml) for 24 hours.
Fig. 3. TRI in placental fibroblast of rhesus monkey following systematic treatment with IFN-a[11]. Note features indistinguishable from the TRI in human calls.
Fig. 4. Paracrystalline array of membranous tubules expanding cisternum of ER in placental capillary endothelium of a rhesus monkey following systematic treatment with IFN-a[11].
Fig. 5. Cytochemical reaction for acetyl cholinesterase[37] in peripheral blood lymphocyte from patient with AIDS and elevated serum IFN-a. Electron-dense deposits highlight the ER, perinuclear envelope (arrow) and region with TRI.

paracrystalline configuration[11].

Ultrastructural Cytochemistry of TRI

Initial cytochemical digestion studies disclosed that TRI contain lipid and trypsin-resistant acidic protein components[77]. Nucleic acid was not detected by selective digestion, ion labelling, or autoradiographic techniques[22,77]. Additional enzyme cytochemical studies now indicate that reactions for several enzymes typically associated with the ER also are positive in regions of TRI (Table 1, Fig. 5), and suggest that ER-associated enzyme proteins must insert into membranes comprising TRI during or after morphogenesis.

Experimental Induction of TRI in Cell Structures

Experimental induction of TRI has been demonstrated in short term cultures of human peripheral blood lymphocytes[17,47], in cultures of continuous lymphoblastoid cell lines (B-cell or T-cell origin), and in cultures of human endothelial cells[12,51]. The initial model for TRI induction involved treatment of lymphoblastoid cell cultures with halogenated pyrimidines[16,24,28], and TRI formation was related to S-phase incorporation of BUdR[28]. Subsequent work demonstrated that the effects observed were secondary to induction of potent lymphoblast IFN-a[70,71,86].

Human Blood Lymphocytes

TRI developed in short term cultures of unstimulated peripheral blood lymphocytes (PBL) exposed to IFN-a. These included both B cells and T cells, as demonstrated by immunoperoxidase staining[32]. Blastogenic doses of either Concanavalin A (Con A) or pokeweed mitogen before IFN-a treatment increased the apparent size and frequency of TRI in thin sections[17,47]. TRI also developed in PBL experimentally incubated with Staphylococcus aureus, Cowan type 1[46] or with UV-inactivated Sendai virus[17]. In each of the latter conditions autogenous IFN-a was produced[17,46].

Human Lymphoblastoid Cell Lines

TRI have been induced in human lymphoblastoid cell lines of diverse origins, including lines which express a B-cell phenotype or a T-cell phenotype[21]. Efforts to induce TRI in the histiocytic cell line U-937[4,68] were unsuccessful[21]. Initial studies with halogenated pyrimidines had demonstrated that neither the source of B cell (neoplastic or non-neoplastic) nor the burden of EB virus genomes were critical factors in TRI induction[22]. This was subsequently confirmed by direct IFN-a treatment of several B cell and T cell lines[21]. Recently, induction of TRI has been observed in two additional B-cell lines (DG-75 and Ramos) which are free of EB virus[2,41] and in a T-cell line (H-9) which carries no known retroviruses[64]. Infection of the latter with HIV-1 exerted no major effect on TRI formation by IFN-a (Orenstein J, Grimley PM, unpublished data).

The earliest formation of TRI has been observed in Daudi lymphoblasts treated for 12 hours with 500 to 1000 IU/ml of IFN-a[21]. In a dose range of 10 to 1000 IU/ml of IFN-a, maximum accumulation of TRI occurs within 48 to 96 hours[21]. Differences in cell line sensitivity have been noted: The B cell lines Daudi, Raji and SB are more sensitive than Namalwa or BJAB, while the T cell lines HSB2 or H-9 are more sensitive than Molt or CCRF-CEM. The monocytic line U-937 is a "non-responder"[21], despite the presence of more numerous type I receptors than Daudi cells (Zoon, KC, personal communication).

Table 1. Cytochemistry of TRI

Selective Fixation	Method	Result
Osmium-ferricyanide[19]		+
$KMnO_4$[74]		+
Selective Staining		
UAc - EDTA (RNA)[77]		-
Ag - proteinate (glycogen)[22]		-
Chromic-acid PTA (mucoprotein)[77]		+
Selective Extraction[74]		
RNAase		-
DNAase		-
Pronase		+
Pepsin (peptide links)		+
Trypsin (basic proteins)		-
Enzyme Reactions		**Results**
Acid phosphatase	59	+
Guanylate cyclase	35	+
Acetyl cholinesterase	37	+
Glucose 6-phosphatase	36	+
Peroxidase	33	-
Catalase	34	-

Sources of previous data are cited following each procedure. References for new experiments are cited under "Method". For these procedures, Daudi cells were treated with 300 IU/ml of IFa for 48 to 72 hours. Approximately 5 x 10^{-6} Daudi cells were sedimented at 400 x g, washed x 1 with ice cold serum free medium, then fixed in ice cold 2% glutaraldehyde and 1% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.2). Duration of fixation was 15 to 20 min for localization of glucose-6-phosphatase and 5 to 8 min for localization of guanylate cyclase or acetyl cholinesterase. After fixation, cells were rinsed x 3 in buffer and stored 4 C for up to 12 hours. Reaction mixtures and incubation procedures are given in references 33-37 and 59.

Lymphocytes from Non-Human Primates

New investigations have included electron microscopy of chimpanzee or rhesus monkey lymphocytes in short term cultures, and of continuous lines of lymphoblasts from the baboon (Papio hamadryas, ATCC CRL 1494, chronically infected with a herpesvirus papio) or old world Stumptail monkey (GM 3442). In chimpanzee or monkey lymphocytes, IFN-a (500 IU/ml for 76-92 hours) induced the loose form of TRI[12], whereas the paracrystalline forms (similar to type in Fig. 4) developed in the baboon and old world monkey cells incubated with IFN-a (100 to 1000 IU/ml for 48-72 hours).

Endothelial Cells

Recent studies of early passage subcultures of well differentiated human endothelial cells demonstrated induction of TRI in endothelial cells of venous (umbilical cord) or arterial (thoracic aorta) origins[12]. Both IFN-a[12] and beta interferon[51] have

proven active on umbilical cord venous endothelium. With IFN-a, TRI developed as early as after 48 hours of treatment and persisted for up to at least seven days of treatment. Apparent frequency and size of the TRI in thin sections generally showed a positive correlation both with the IFN-a concentration in a range of 500 to 2000 IU/ml, and the duration of exposure. In contrast to findings in rhesus monkeys treated systemically with IFN-a[11], (see below), the TRI in human endothelial cells were never paracrystalline.

Current Limitations of Quantitation of TRI

A persistent impediment to quantitative investigations of TRI is the lack of discrete immunocytochemical or biochemical markers. Although the aggregates of TRI can measure up to 2.5 micrometers maximum dimension, there is yet no discrete technique for recognizing them in the range of light optical resolution. Transmission electron microscopy remains the only tool for definitive and quantitative identification. Both experimental and clinical analyses therefore must rely on labor intensive thin section counts which are subject to binomial statistics[18,70]. At least 200 thin sections must be examined per experimental time point to determine binomially significant differences, preferably with serial sets of data points, as in dose effect or kinetic studies[12,21,28,70].

TRI Formation During Clinical IFN-a Therapy

Clinical trials of IFN-a therapy and pharmacologic tests have provided useful materials for investigating TRI formation in intact organisms. This is important because an extensive body of experimental veterinary and clinical literature (reviewed in reference 22), previously had illustrated TRI in a variety of cell types and a number of experimental or disease conditions. Viral infection appeared to be a major common denominator[22,28,63] and the possibility of a relatively low molecular weight serum factor was suspected in the pathogenesis of TRI in congenital autoimmune disease[43,50].

Peripheral Blood Mononuclear Cells (PBMC)

Several series of patients with chronic viral infections or neoplasia have been systematically treated with high doses of IFN-a[10,66,67]. In chronic hepatitis type B and in hairy cell leukemia, TRI have been noted in circulating lymphocytes and in other PBMC[17,26]. In PBMC samples from patients with chronic hepatitis, immunoperoxidase studies to identify T-cells, B-cells, NK-cells and monocytes disclosed that TRI formed in all subsets; there appeared to be no preferential target population[17]. Comprehensive pre-treatment and post-treatment quantitative evaluations were conducted: pellets of PBMC were examined under standard conditions. TRI developed within 24 hours after commencement of treatment, persisted and increased slightly during a two week regimen of therapy (up to 10% of sections), then entirely disappeared within 3-11 days after termination of therapy.

Endothelial cells

In contrast to pellets of PBMC which lend themselves to random thin section examination and statistical evaluation of morphological data, the variable orientation of flat endothelial cells lining tissue capillaries complicates quantitative analyses. Moreover, serial biopsies of capillary rich tissues such as liver or renal glomeruli may not be medically indicated during IFN-a therapy. Despite these problems, TRI were detected in hepatic endothelial and Kupffer cells in liver biopsies from two patients undergoing IFN-a therapy trials[80], and in endothelial cells of a teratoma from a patient treated with IFN-a[72].

Studies after recombinant human IFN-a treatment of pregnant rhesus monkeys provided a unique opportunity to examine effects on the blood vasculature[11]: electron microscopy of placental chronic villi disclosed abundant TRI both in capillary endothelium (Fig. 4) and in the stromal Hofbauer cells and fibroblasts (Fig. 3); TRI were not found in control specimens.

Interferon toxicity

TRI formation in vivo may be associated with cytotoxic viral infections[23,38]; however, there has been no direct evidence of toxic clinical effects on the mononuclear or endothelial cell targets of TRI formation[11,17]. Clinical and in vitro studies of TRI morphogenesis also have provided no indication that the formation of TRI heralds cytotoxicity. TRI can form during active growth of lymphoblastoid cell cultures[21] and there was no morphologic evidence of cytotoxicity in endothelial cell cultures[12].

Multiple converging profiles of endoplasmic reticulum develop in the hepatic cells of fetal mice after hepatotoxic exposure to mouse IFN-a and have been related to TRI[56,57], but intracisternal tubular structures evidently do not develop. Cylindrical confronting cisternae (CCC) caused by internal fusions of the ER[3,44,75] have also been associated with clinically high levels of IFN-a[60,82], but their specificity and reversibility is not certain[44]. Experimental considerations of IFN-a and a long duration of incubation are required (1000 IU/72 hours) to produce CCC in Daudi cells[3]. In our experience these conditions are irreversibly cytotoxic. In AIDS, CCC are considered an ultrastructural marker[3,60,82] and occur in conjunction with TRI[3,60,82]; however, the clinical development of CCC is associated with terminal disease[60].

Experimental Modulation of TRI Formation

Studies with metabolic inhibitors in the Daudi lymphoblast model have provided a few clues to molecular mechanisms involved in TRI morphogenesis. Morphogenesis is totally inhibited when actinomycin D is added at up to 4 hours after IFN-a and the frequency of TRI is reduced when actinomycin D is added before 12 hours[21]. These findings indicate that TRI formation depends upon new RNA transcription. Inhibition of protein synthesis by cycloheximide before 12 hours of IFN-a incubation also blocks TRI formation; however after 12 hours of IFN-a incubation, TRI formation is not significantly blocked by cycloheximide[21]. The results suggest that TRI morphogenesis may depend upon protein synthesized earlier than 12 hours.

Feedback inhibition of DNA replication and blockage of S-phase by excess thymidine[88] does not block TRI formation; indeed, the proportion of TRI increased when growth of Raji lymphoblastoid cells was slowed by thymidine synchronization prior to addition of IFN-a[21]. As discussed above, halogenated pyrimidines (BUdR, IUdR) actually induce the formation of TRI in a number of B cell lines[16,28], presumably due to secondary stimulation of autogenous

IFN-a production[79,86].

It was of particular interest to test experimental effects of cytoskeletal paralysis, since the cytoskeleton is intimately involved in membrane flow and translocation[55] and also has been implicated in IFN-a signal transduction[6]. Poisons included deuterium oxide (microtubule stabilizer)[49,84], colchicine or vinblastine (microtubule depolymerizers)[84], and cytochalasin B (microfilament depolymerizer)[29]. For present studies, these were added before, with, or after IFN-a (Table 2). Deuterium oxide (D_2O) completely blocked, and the other drugs partially inhibited TRI morphogenesis. These results indicate that integrity of cytoskeletal system is essential for the TRI development in Daudi cells. Results with D_2O are especially important, since the inhibition of TRI formation can thus be dissociated from some other biological effects of IFN-a (see below).

Table 2. Effect of Cytoskeletal Poisons on TRI Formation

Inhibition#	Concentration	Time added*	TRI at 48 h
Colchicine	10^{-6}M	-2 h	+
		0 h	+
		8 h	+
Vinblastine	10^{-6}M	0 h	+
Cytochalasin B	10^{-6}M	0 h	+
D_2O	40%	-2 h	-
		0 h	-

#All inhibitors purchased from Sigma Chemical Co. St. Louis, MO. D_2O prepared in 10 X RPMI 1640 (Gibco, Grand Island, NY) and diluted to appropriate concentration.

*Time added after IFN-a (300 IU/ml). At this concentration TRI were induced in up to 10% of cell sections in positive controls.

Relation of TRI induction to Biological Effects of IFN-a

Many medically significant biologic effects of IFN-a occur within 12 hours after incubation. In contrast, the morphogenesis of TRI does not become evident until 12 hours, and then only at relatively high concentrations of IFN-a[21]. Inhibitor studies, reviewed above, suggested that TRI morphogenesis depends upon a prior accumulation of structural proteins or of enzymes, but the exact sequence of pre-morphogenetic events remains uncertain.

TRI and Anti-Viral Activity

The effect of IFN-a on establishment of an antiviral state is nearly universal and not species restricted. The virus growth inhibition effect is evident as early as 4 hours after incubation[1,74]. While almost all human cell lines with type 1 receptors are sensitive to an antiviral effect of IFN-a, development of TRI is evidently not as universal: for example, TRI were not found in A549 human lung cancer cells or trisomy 21 fibroblasts which are endowed with abundant type 1 receptors[21,46,69].

TRI in NK effector cells

TRI can be induced in human NK cells. This was recently demonstrated by immunocytochemical labelling with Leu 11a+ or Leu 7 antibodies[31,32,34]; however, the time scale for stimulation of NK cell effector functions (binding to and lysis of target cells) can precede TRI morphogenesis[9,62].

Growth Inhibition and TRI

Compared to either antiviral activity or NK effector modulation, growth inhibition by IFN-a is a relatively late effect and closely parallels TRI formation both in highly sensitive Daudi lymphoblastoid cells and endothelial cell cultures[12,21]. As discussed above, however, the anti-proliferative and morphogenic activities did not prove to be consistently linked: TRI also developed (but to a lesser extent) in a Daudi mutant clone which selectively resists the anti-proliferative activity of IFN-a[21,53,83]. Conversely, we have found that treatment with 40% D_20, which inhibits TRI morphogenesis, does not prevent the IFN-a antiproliferative or 2'-5' A synthetase induction activities (Tables 2, 3). Again, this demonstrated that the phenomena could be disassociated. Such results are not unexpected in view of current hypotheses which implicate multiple signal transduction pathways to explain the pleiotropic biologic actions of IFN-a[13,53] after binding to a common set of type I receptors[69].

Table 3. Effects of D_2O (40%) on growth and 2'-5' AS induction of Daudi cells treated with IFN-a (300 IU/ml)

Time*	Growth rate@ (72 h)	2'-5' AS# (24 h)
-2 h	39%	7 to 11 x
0 h	70%	7 to 11 x
8 h	55%	not tested

*Time after IFN-a added.

@Compared to D_2O controls at 72 h (mean of 2 experiments at each time point).

#Specific activity of 2'-5' A synthetase measured as product of 2'-5' A/mg protein/h compared to control values for D_2O treated or untreated cells without IFN-a. Methods were described previously[21]. Control range of 2'-5' A produced in assay: 2.6 to 4.0 μMol/mg/h.

TRI and 2'-5' A Synthetase Induction

Induction of 2'-5' A synthetase is the only IFN-a-associated phenomenon which thus far has not been uncoupled from TRI induction. Elevated 2'-5' A synthetase was noted both in lymphoblastoid and endothelial cell cultures which develop TRI[12,21]. Actinomycin D and cycloheximide diminished induction of 2'-5' A synthetase in parallel with inhibition of TRI[21]. Although smooth cytomembrane proliferations in the liver[25] and in Chinese hamster endothelial cells previously have been associated with excess enzyme induction[30,61], the 2'-5' A system is not membrane dependent[48] and the relationship to

TRI remains unclear.

Thymidine uptake

IFN-a blocks uptake of thymidine into many cell types. This appears to be a direct effect on thymidine kinase, rather than a consequence of growth inhibition[14]. We have recently observed a similar effect on amino acid and galactose transport into Daudi cells after 12-24 hours incubation in IFN-a, 100 IU/ml (Rupp, B, Grimley, PM, unpublished data). These phenomena probably are independent of TRI formation. Feedback inhibition of DNA biosynthesis by excess thymidine[88] also had no effect on TRI morphogenesis in Daudi cells.

Correlations of TRI Morphogenesis with Effects on Macromolecular Metabolism

Protein biosynthesis

Studies of lymphoblasts treated with BUdR indicated a shift in protein biosynthesis during TRI formation[71]. Gel electrophoretic analysis of hairy leukemic cells which develop TRI after IFN-a treatment have shown induction of up to 16 proteins[75,76]. Similar results have been reported with Daudi cells after IFN-a treatment[53]. Enzyme cytochemical data[79] indicate that TRI membranes are associated with protein; however, the specificity and relation to induction of specific proteins by IFN-a remains to be determined.

Glycoprotein biosynthesis

Formation of massive TRI in the ER raises questions regarding their possible functional effect on cytosecretory activities. A role of TRI in immunoglobulin secretion was formerly postulated[87], but would not explain the development of TRI in T cells or endothelium. Daudi lymphoblastoid cells which form abundant TRI also secrete IgM[40]; and they are a promising model for testing effects of TRI on the cytosecretory process. It has been shown that biosynthesis of IgM in Daudi cells diminishes during IFN-a treatment[27], but an effect of TRI morphogenesis on glycoprotein translocation through the Golgi[73] was not thereby excluded. In preliminary studies, we have observed a reduction of total cellular IgM in extracts of Daudi cells treated for 24 or 48 hours with IFN-a (Fig. 6). At up to 24 hours, we could discern no general effect of IFN-a on the pattern of glycoprotein biosynthesis in extracts of cells analyzed by gradient gel electrophoresis after labelling with ^{3}H-galactose (Fig. 7). After 24 hours, uptake of ^{3}H-galactose was almost totally blocked (Fig. 7); but Western blots for total IgM (Fig. 6) failed to suggest any correlation of TRI with accumulation of unlabelled glycoprotein. This is consistent with immunofluorescence evidence of sIgM down-regulation[81].

Lipid Biosynthesis

The role of lipids in TRI appears self-evident from ultrastructural and cytochemical evidence. The preservation of TRI membranes after $KMnO_4$ oxidation is particularly striking (Fig. 8) and favors the concept of a phospholipid core structure[77]. In neonatal mice treated with interferon, there was a marked increase in hepatic triglycerides accompanied by a decrease in phospholipids[89], and this may be the counterpart of morphologic changes in the endoplasmic reticulum (see references 56, 57). Using Daudi cells treated with IFN-a, we found that total uptake of ^{3}H-glycerol is not decreased by IFN-a. This contrasted to the diminished uptake of ^{3}H-TdR, ^{3}H-galactose and ^{3}H-amino acids (see above). Preliminary results of lipid extractions and high resolution thin layer chromatography[58] further indicated that specific incorporation of ^{3}H-glycerol into phosphatidyl choline increases two-fold between 46-54 hours, at a time when TRI are accumulating (Table 4).

Table 4. Thin layer chromatographic analysis of Daudi cells labelled with ^{3}H-glycerol* at 46-54 hours

Treatment	CPM/mg dry weight**	Phosphatidyl Choline specific activity@ (cpm/pMol)
None	319	2.0
IFa 100 IU/ml#	980	4.3
IFa 1000 IU/ml#	899	5.1
3mM TdR	320	2.2

#Ultrapure leukocyte interferon (Interferon Sciences, New Brunswick, NJ).

*Specific activity 33.7Ci/mM, concentration 5 μCi/ml

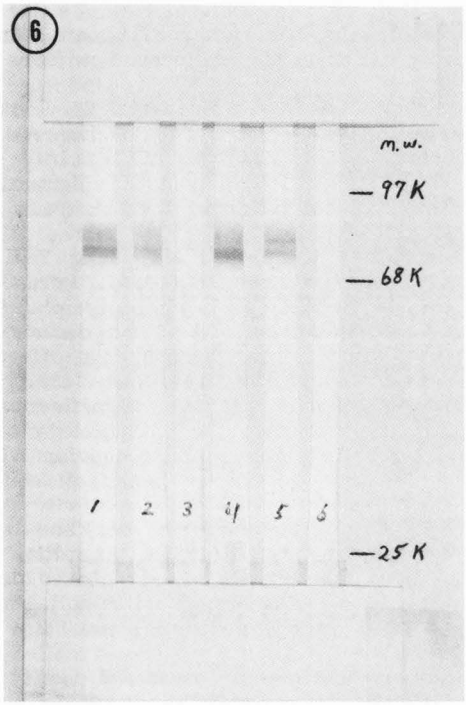
**Pellet desiccated prior to extraction by Harth method (58)

@Phospholipid bands identified by molybdenum blue staining and quantitated by scanning densitometry (58). Radioactivity counted after scraping of bands into organic scintillation fluid.

Clinical Pathogenesis of TRI

Despite the technical demands of TRI quantitation in ultrathin sections, detection of TRI in peripheral blood mononuclear cells has proven to be a valid clinical parameter in evaluating groups of patients with persistently abnormal endogenous elevations of serum IFN-a (e.g., AIDS, systemic lupus erythematosus)[19,20,42,60]. In AIDS, appearance of TRI correlates strongly with disease progression, reduction in T4 cells and production of endogenous acid-labile IFN-a[19,20,60,65]. In systemic lupus erythematosus, both prospective and retrospective analysis have disclosed a positive correlation of TRI formation with abnormal serum levels of IFN-a[19,42].

Disseminated TRI are most frequent in diseases with high levels of endogenous interferon production[44,45]; however, TRI have also been observed in a number of other conditions, without measurable levels of serum IFN-a[85]. Mechanisms for pathogenesis of TRI probably include virus infections with localized paracrine or autocrine IFa production[7-9,54]. TRI are common in fetuses from gestations complicated by rubella infection[38], and may be associated with interferonogenic togavirus infections under experimental conditions[15]. Cells chronically infected with HIV-1 may induce formation of the acid labile alpha interferon prevalent in AIDS[5]. A role of endotoxin in stimulation of autogenous interferon production



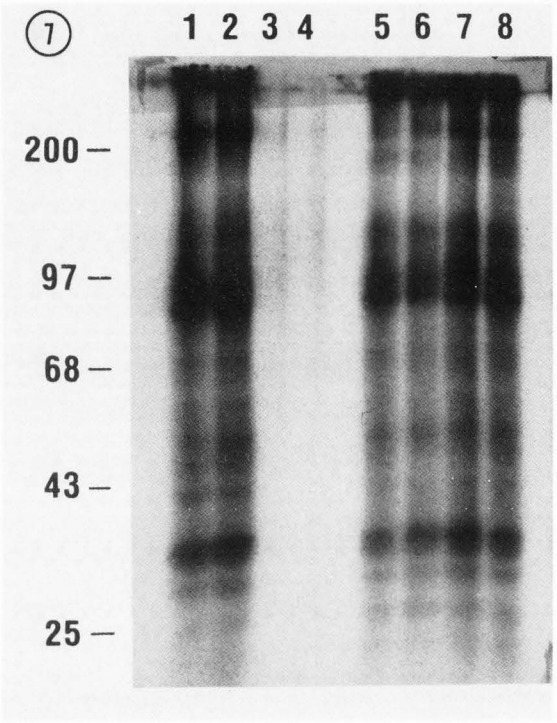


Fig. 6. Western blot for IgM. Cells were extracted with 0.5% Nonidet P-40 buffer and 2 mg of protein were applied to each 0.5 cm lane on a 6-12% SDS gradient gel for electrophoresis. A nitrocellulose blot was blocked with milk reagent, then reacted with biotinylated goat polyclonal anti-IgM, and developed with the Vector ABC method. Lanes 1 and 4: Daudi control cells at 24 and 48 hours. Lanes 2 and 5: Daudi cells treated with 500 IU/ml of IFN-a for 24 and 48 hours. Lanes 3 and 6: H-9 (T-cell) control cells at 24 and 48 hours.

Fig. 7. SDS 6-12% gradient gel electrophoresis of Daudi cells stabilized in Laemmeli buffer after 8 hours labelling with ^{3}H-galactose at 46 hours (lane 1 - 4) or 16 hours (lane 5 - 8). Control samples (lanes 2, 6) and samples treated with 3mM thymidine to inhibit growth (lanes 1, 5) were compared with samples treated with IFN-a 100 IU/ml (lanes 3, 7) or IFN-a 1000 IU/ml (Lanes 4, 8).

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was recently demonstrated in NK cells and associated with TRI formation[48]. Thus, the development of TRI in human blood cells or solid tissues in infections, auto-immune diseases and neoplasias may be stimulated by a wide variety of pathogenetic events predisposing to local increases of interferon[7,39,52].

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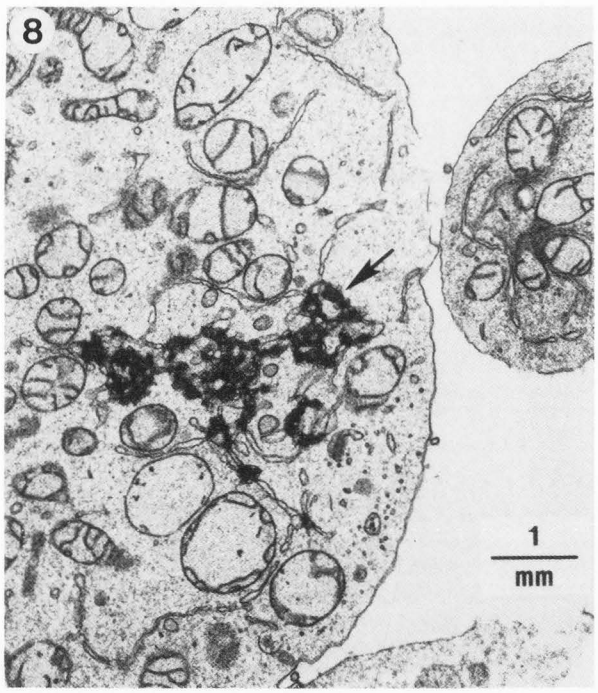

Fig. 8. Daudi cells with TRI fixed in 0.6% $KMnO_4$[77]. TRI and cytomembranes are highlighted. Ribosomes and most proteins are extracted.

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Discussion with Reviewers

H. Gamliel: Using immunoelectron microscopy we recorded changes in membrane antigens and receptors of Daudi cells as early as 2 h after incubation with 1000 U/ml of IFN-a (see Gamliel H, Gurfel D, Wu SH, Golomb HM, Interferon induced alterations in hairy cells - a review, Scanning Microsc 2: 485-492, 1988). You state that TRI are detected only after 12 hours. It might be that these two types of types of changes are totally unrelated, but could you exclude the possibility that TRI are seen later because they are not induced by IFN-a itself, but rather by some other factor existing in the cultures. Did you use gamma interferon, B-cell growth factor, anti-IFN-a or other controls to prove that TRI are directly induced by IFN-a?

Authors: This raises an important, but complex question. We previously learned that the delayed, S-phase effect of halogenated pyrimidines on TRI induction[28] was actually due to a secondary stimulation of interferon production in the cultures[86]. Thus, it is perfectly logical to inquire whether the delayed effect of IFN-a itself reflects just another level in a cascade of growth factors leading to TRI formation. In our view, a cascade of subcellular biochemical events (such as induction of 2'-5' A synthetase) probably does precede TRI formation and account for the delay in their morphogenesis, but this is not evidently due to the "feedback" and production of other extracellular bioregulators such as gamma interferon. This conclusion is based upon experimental induction

of TRI in T-cells and endothelial cells which have different growth factor binding affinities and sensitivities as compared to B-cells, and also upon failure thus far to induce TRI with gamma interferon or with IL-2, either directly or indirectly, by exposure of lymphocytes to lectins[17]. Interestingly, beta-interferon, which shares type I receptors with IFN-a, does induce TRI. With regard to specificity, we have used pure recombinant forms of IFN-a and blocked TRI induction with actinomycin D (this indicates involvement of new mRNA transcription).

H. Gamliel: Studying TRI by means of transmission electron microscopy bears the disadvantage of screening only a thin slice of each cell. In hairy cell leukemia we were unable to correctly determine the percentages of cells displaying TRI formation. Besides time-consuming serial sectioning, are there other methods available for quantitating TRI in mixed cell population?
Authors: A simpler method for quantitation of TRI or component product has been a major objective of our research, but we have thus far found no specific radiolabel, immunolabel, or stain which can be detected by light optics. Conventional tissue processing for light microscopy is unsatisfactory, since lipids are extracted during embedding. Fixation of cells with $KMnO_4$ offers a useful alternative for electron microscopic screening, since the TRI are thus highlighted (Fig. 8); but the counting of thin sections is still tedious. Incidentally, we prefer step rather than serial sections, since the significance of differences in TRI counts is a binomial statistic. Based upon the assumption of round cells, we previously estimated broad ranges for significant differences in TRI counts when at least 200 random thin sections of separate cells were counted[28]. TRI counts of serial samples or multiple patient samples have been successfully correlated with clinical data in double blind studies[20,42,60], and there have been good correlations in studies of IFN-a therapy[17,26]. Despite these results, we agree that development of a more sensitive, quantitative detection method would be more practical and more widely applicable in clinical diagnosis, follow-up of disease progression or evaluation of therapies.

A. Hiraoka: The authors and their colleagues suggested that TRI contain an acidic protein component[77], and inhibitor tests indicated that TRI formation depends upon protein synthesis[21]. Among the numerous complex changes induced by interferon treatment, overall inhibition of protein synthesis and increased synthesis of a specific group of proteins has been noted. An analysis of protein synthesis and patterns during TRI formation might aid in biochemical identification of TRI components. Have you ever studied this relationship?
Authors: This is essential work which needs to be pursued. As you point out, up to 16 induced proteins have been detected in extracts of leukemic cells after IFN-a treatment[53,75,76]. In patients with hairy cell leukemia, protein induction by IFN-a can continue for many days[76] and parallel the increase of TRI (Hiraoka A, Rosner MC, Golomb HM, In vitro response of cells from three patients with hairy cell leukemia to a recombinant leukocyte interferon. Virchows Arch. [B] 49: 73-82, 1985). One approach might be to compare patterns of protein induction in different cell lines which respond to interferon, but only some of which produce TRI. Another approach would be to analyze cell fractions. We have begun some preliminary tests, but our main emphasis recently has been on the lipid studies.

J. Moss: The effect of interferon on lipid biosynthesis and thus membrane integrity does seem to contribute to the formation of endoplasmic reticulum abnormalities. It has been suggested that the interferon-induced tubular aggregates seen in neonatal mice are the morphologic counterparts of biochemical abnormalities of lipid biosynthesis[56].
Authors: We certainly agree with this point; however, in contrast to the results from experimental studies of suckling mice[89]; our data suggests that TRI in Daudi cells may be associated with increased phospholipid biosynthesis. As with CCC (see below), there may be more than one ER membrane abnormality induced by IFN-a, perhaps depending on dose, duration, or species.

H. Gamliel: You mention that in AIDS, CCC occur in conjunction with TRI. Are the CCC linked physically to TRI or do they just occur in the same cell with no structural connections?
Authors: CCC represent abnormal cylindrical formations of the ER which appear to result from prolonged or excessive IFN-a exposure[3,44]. In AIDS, CCC appear later than TRI and suggest a poor prognosis (Orenstein JM, Preble OT, Kind P, Schulof R. The relationship of serum alpha-interferon and ultrastructural markers in HIV-seropositive individuals. Ultrastruct. Pathol. 11: 673-679, 1987). Ultrastructurally, TRI and CCC sometimes form in the same cells[44], and they can be entrapped within CCC, presumably because they both arise from the ER (Kostianovsky M, Lang Y-H, Grimley PM. Ultrastructural and immunoelectron microscopic studies of cells with abnormal cytoplasmic inclusions in patients with AIDS. AIDS Res. 1: 181-196, 1984).

H. Gamliel: The paracrystalline structures you mention are quite different from the "classical" TRI. Why are they placed in the same category? The fact that both develop in response to human IFN-a treatment does not necessarily prove them to be identical entities.
Authors: We long suspected a similarity of "classical" TRI and the paracrystalline or "geometric" TRI and hypothesized a difference in tubule packing; the tubule diameters are similar[22,79]. It is now evident that both types of structures are induced by IFN-a; however, only the "classical" form occurs in human tissues. In the rhesus monkey, both forms of TRI were observed after systemic IFN-a treatment[11] and this appeared to depend upon cell type (classical form only in endothelium, paracrystalline forms in fibroblasts and syncytial trophoblasts). Work in progress (D. Feldman and P. Grimley, unpublished) shows that both forms may be induced in lymphoblast cultures of subhuman primate origin. We agree that such observations "beg the question" until a biochemical proof of identity becomes available. As in many other morphologic studies, our interpretations are based upon good circumstantial evidence.