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Differential expression patterns of type I interferon subtypes in mouse embryo fibroblasts: influence of genotype and viral inducer

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Abstract

Primary mouse embryo fibroblasts from 4 strains of mice (BALB/c, C57Bl/6, B6.C-*H-28*^{c} and CBA) were infected with either Newcastle disease virus or murine cytomegalovirus. The time course of the total type I interferon response was assessed and the presence of individual subtypes determined. The total type I interferon produced was titrated using the cytopathic effect reduction assay and the relative levels of type I interferon subtypes expressed (α1, α4, α5, α6 and β) were evaluated using a reverse transcription-polymerase chain reaction-based technique. In general, the patterns of type I interferon subtypes expressed appeared to be determined by the strain of mouse cells used rather than the inducing virus. However, the overall titre of type I interferons produced in response to a given virus was quite uniform across the strains of mice from which the mouse embryo fibroblasts were derived regardless of the subtype expression pattern. The latter observation fits the proposition that "cross-talk" or feedback between the type I interferon genes and their products is occurring and that the inducer determines the level of response.

Key words: Type I murine interferon response; Genotype; Viral inducer; Reverse transcription-polymerase chain reaction

1. Introduction

The interferons (IFNs) are proteins found in all vertebrates and their production

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is a cellular response to foreign antigens, nucleic acids and cells. The IFNs are subdivided into type I IFNs (IFN-α, IFN-β and IFN-ω), which are usually acid (pH 2) stable, and type II (IFN-γ), which is generally acid (pH 2) labile (Pestka and Baron, 1981). The IFNs-α in all mammalian species are encoded by a family of closely related genes (Weissman and Weber, 1986). In the human, there are at least 14 IFN-α subtypes and, in the mouse, there are 11 known IFN-α subtypes. However, there is only a single gene coding for IFN-β in both humans and mice. IFNs-ω are absent from mice and are represented by 6 genes in humans, however all but 1 appear to be pseudogenes.

Although the IFNs, in general, have a multitude of diverse biological activities such as antiviral (AV), antiproliferative (AP) and immunomodulatory activities, variations in biological activities between the numerous closely related IFN-α subtypes have been reported. For example the human (Hu) IFN-α1 has potent AV and AP activities but it lacks the ability to boost human natural killer cell activity which is a normal property of IFN-α subtypes (Ortaldo et al., 1984). HuIFNα1 and HuIFN-α8 differ in their AV potency by a factor of 330 (Rubinstein, 1987) and other in vitro differences include murine (Mu) IFN-α4 which is 20 times more potent in inhibiting the growth of an erythroleukemic cell line than MuIFN-α1 (Swarninathan et al., 1992). Besides differences in in vitro biological activities, individual IFN-α subtypes have been reported to be expressed to different extents in a given cell type (Hiscott et al., 1984) and the production of certain HuIFN-α subtypes are also selectively enhanced in leucocytes pretreated with IFN (Goren et al., 1986). It has been suggested that these variations in subtype expression patterns may be due to the subtypes having different roles in vivo (Finter, 1991).

In this study, a recently developed reverse transcription-polymerase chain reaction (RT-PCR)-based technique (Lai et al., 1994) was used to compare the relative levels of expression of four major MuIFN-α subtypes and MuIFN-β following Newcastle disease virus (NDV) or murine cytomegalovirus (MCMV) infection of mouse embryo fibroblasts (MEFs) derived from BALB/c, C57Bl/6, B6.C-*H-28*^{c} and CBA mice. The relative influences of genotype and viral inducer on the patterns of type I IFN expression could then be assessed in these primary cells.

2. Materials and methods

2.1. Cell cultures

MEFs were derived from 15–17 day old embryos of BALB/c (*H-2*^{d}), C57Bl/6 (*H-2*^{b}), CBA (*H-2*^{k}) and B6.C-*H-28*^{c}, a congenic strain which differs from C57Bl/6 by a BALB/c allele at the *If-1* locus (DeMaeyer et al., 1975), mice by trypsin dispersion and were cultured in Eagle's minimal essential medium (MEM) with Earle's salts, glutamine and 10% fetal bovine serum. Cultures were used between passages 4–6. L929 cells were grown in monolayers in Eagle's MEM supplemented with 10% fetal bovine serum.

2.2. Virus stock preparation

Stocks of MCMV were prepared from the salivary gland of weanling female BALB/c mice infected intraperitoneally with 8×10^3 plaque forming units (pfu)/mouse of the Smith strain of MCMV. Tissue culture-derived MCMV was prepared by passaging the salivary gland-derived-MCMV once in BALB/c MEFs. MCMV titres were assayed as pfu on day 5 post-infection (p.i.) in BALB/c MEFs using a methyl cellulose overlay (Allan and Shellam, 1984). Stocks of NDV were prepared by inoculating a local NDV isolate into the allantoic fluid of 9–11-day-old embryonated chicken eggs. The allantoic fluid was harvested and clarified 4 days p.i. NDV titres were assayed as 50% egg infectious dose (EID_{50}). Encephalomyocarditis virus (EMCV) was propagated in L929 cells.

2.3. MCMV and NDV infection of MEFs

Confluent monolayers of MEFs at between passages 4–6 were washed in mouse osmolarity-buffered saline (MOBS) and inoculated with either MCMV in 1 ml MOBS at a multiplicity of infection of 0.4 or with NDV in 1 ml MOBS at 6 EID_{50}/cell. After 1 h incubation at 37°C, the inoculum was removed, 10 ml of MEM with 5% fetal bovine serum were added and the MEFs were incubated at 37°C. At 0, 4, 6, 8, 10, 12, 16, 20, 24, 48 and 72 h p.i., the supernatant was harvested for IFN assay and the cells were washed twice with MOBS and then processed for RNA extraction using RNAzol^{TM} B (BIOTECX, Texas, USA) as detailed in Lai et al. (1994). Uninfected control MEFS were inoculated with 1 ml MOBS and processed in a similar manner.

2.4. Type I interferon detection and identification

The RT-PCR-based technique for the detection and identification of type I IFN subtypes is detailed in Lai et al. (1994). In brief, it involved the following steps: (1) the RT of total cytoplasmic RNA isolated from control and virus-infected MEFs using oligo$(dT)_{12-18}$ as the primers; (2) the amplification of any cDNA produced by PCR using the primers 5′-TCTCTCCTGCCTGAAGGAC-3′ (upstream primer, corresponding to the region coding for amino acids 26–32 of MuIFNs-α) and 5′-ACACAGTGATCCTGTGGAA-3′ (downstream primer, corresponding to the region coding for amino acids 124–130 of MuIFNs-α). This results in the amplification of a 308 b.p. fragment from all known MuIFN-α subtypes. Another set of primers 5′-CAGCTCCAAGAAAGGACGAA-3′ (upstream primer, corresponding to the region coding for amino acids 7–13 of MuIFN-β) and 5′-GTAGCTGTTGTACTTCATGAG-3′ (downstream primer, corresponding to region coding for amino acids 130–136 of MuIFN-β) were used for the amplification of a 390 b.p. fragment of MuIFN-β. The parameters used for amplification of IFNs-α and β were denaturation at 95°C for 5 min, annealing at 55°C for 2 min and extension at 70°C for 4 min for the first cycle, followed by denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 70°C for 2 min for another 29 cycles and (3) the identification

Table 1
Sequences, size, T_m and location of the identifying oligonucleotides (IP) for MuIFNs-α1, α4, α5 and α6

Designation for IP	Sequences of oligonucleotides used as specific hybridisation probes for identification of type 1 MuIFN subtypes	Size in b.p.	T_m in °C	Location of oligonucleotides in the *n.t. sequence
IP-α1	5'-A TTT CCC CTG ACC CAG GAA GAT G-3'	23	70	324–346
IP-α4	5'-CC TGT GTG ATG CAG GAA CCT CC-3'	22	70	293–329
IP-α5	5'-T GAA GTC CAT CAG CAG CTC AAT-3'	22	70	261–282
IP-α6	5'-CAG GTA GAG ATA CAG GCA CTT CC-3'	23	70	307–329

*n.t. – nucleotide.

of the amplified individual MuIFN-α subtypes by hybridisation of the PCR products to [γ-^{32}P]ATP-labelled oligonucleotides specific to MuIFN-α subtypes. These oligonucleotides are referred to as identifying primers (IP) and they are designated IP-α1, IP-α4, IP-α5 and IP-α6, specific for identifying MuIFN-α1, α4, α5 and α6, respectively. The lengths, melting temperatures (T_m) and locations of the identifying oligonucleotides are presented in Table 1.

2.5. Type I IFN assay

Supernatants from NDV and MCMV-infected MEFs and uninfected controls were harvested and dialysed against 0.1 M glycine HCl (pH 2) for 2–5 days to eliminate the activities of the virus and acid-labile IFNs and then against MOBS (pH 7.2) for another 2 days. All dialysis was conducted at 4°C. The dialysed supernatants were passed through 0.45 μm millipore filter. Acid-stable total type I IFNs present were titrated using the method of cytopathic effect (CPE) reduction in L-cells infected with EMCV (Jilbert et al., 1986). The mouse IFN-α from Lee Biomolecular (San Diego, USA) was used as a standard in the assay.

3. Results

3.1. Total acid-stable type I IFN response in NDV-infected MEFs

The supernatants of uninfected and NDV-infected MEFs from BALB/c, C57Bl/6, B6.C-*H-28*^{c} and CBA mice were dialysed against glycine HCl (pH 2) for 5 days and then assayed in triplicate using the CPE reduction bioassay. Acid-stable type I IFN was not detected in any of the uninfected MEFs. The titers of the acid-stable type I IFN produced in NDV-infected MEFs from all 4 strains of mice over a 72 h time course are shown in Fig. 1. The overall kinetics of type I IFN production as well as the total type I IFN titers produced in NDV-infected MEFs from all 4 strains of mice were similar. In general, the IFN titers peaked at about 10 h p.i. and were maintained throughout the rest of the time course.

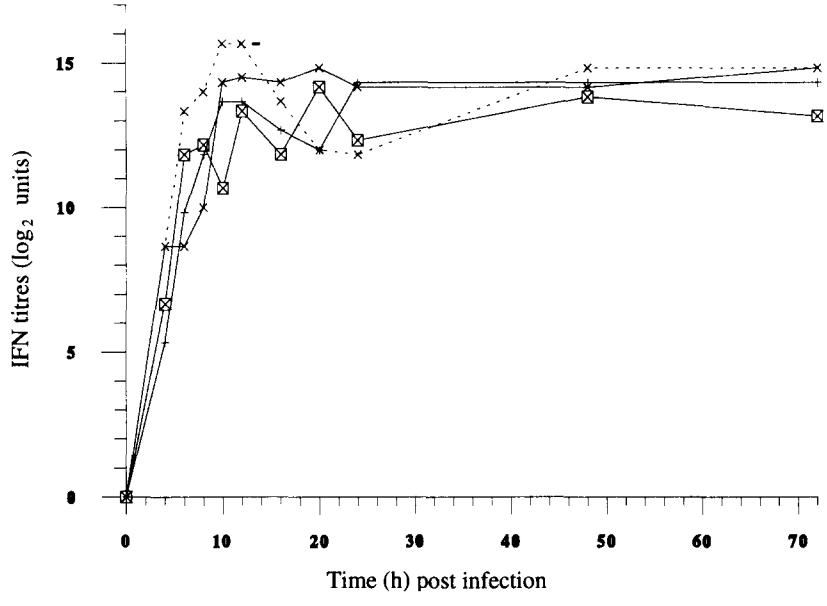

Fig. 1. Graph showing total acid-stable type I MuIFN titers in supernatants of NDV-infected MEFs from BALB/c (–+–), C57Bl/6 (—×—) B6.C-*H-28*^{c} (- - ×- - -) and CBA (—⊠—) mice at various times (h) p.i.

3.2. Type I IFN subtype analysis in NDV-infected MEFs

After removal of the supernatant for total type I IFN titration described above, monolayers of control and NDV-infected MEFs from the 4 strains of mice were washed in MOBS. Total cytoplasmic RNA was then extracted from the MEFs over the same 72 h time course. These infection experiments were performed at least twice as independent runs. The total cytoplasmic RNA was then DNAase-treated to remove any contaminating genomic DNA. The absence of contaminating genomic DNA was then confirmed by direct PCR of the DNAase-treated total cytoplasmic RNA (see also Lai et al., 1994). Complete absence of any amplification product was a routine requirement before proceeding with any further analysis of a given RNA sample (data not shown). Complementary DNA (cDNA) was synthesised in 2 independently controlled experiments from the RNA using oligo$(dT)_{12-18}$ as primers and the resulting cDNA was then amplified by PCR using the 2 sets of primers described in Section 2 using the conditions detailed in Lai et al. (1994). The expression of MuIFN-α and MuIFN-β, confirmed by the presence of a 308 b.p. and 390 b.p. fragment, respectively, upon electrophoresis of the PCR products, was detected in NDV-infected MEFs from all 4 different strains of mice (data not shown). In contrast, no band was detected in the control uninfected samples over the same 72 h time course. 10 and 1 μl aliquots of pooled PCR products from the independent RT reactions were amplified using the set of primers specific for MuIFNs-α and dot blotted onto nitrocellulose membranes, as shown in Fig. 2 (panels a and d). The identifying oligonucleotides (IP-α1 IP-α4, IP-α5 and IP-α6) were then labelled with

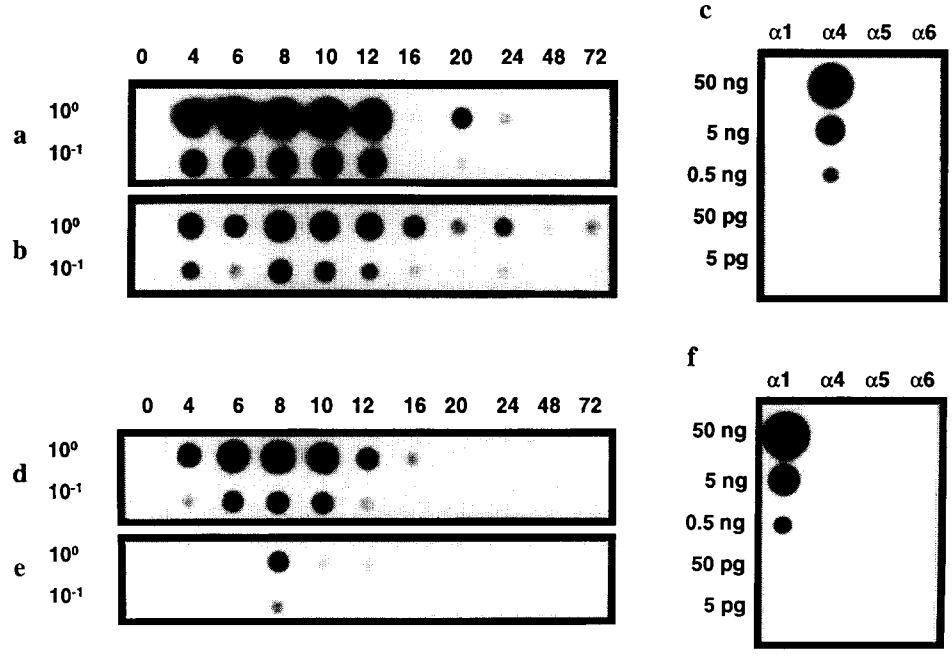

Fig. 2. Autoradiographs showing results of dot blot hybridisation of pooled PCR products derived from virus-infected MEFs from BALB/c mice (2 separate infection experiments). The PCR products were generated in separate RT reactions for each time point using the set of primers specific for MuIFNs-α. Pooled PCR products were hybridised with [γ^{32}P]ATP-labelled-IP-α1 and IP-α4. The final concentration of the [γ^{32}P]ATP-labelled-oligonucleotide used was 10^6 d.p.m./ml of hybridisation solution. Panel a shows the results of the hybridisation using 10 μl (upper row) and 1 μl (lower row) of pooled PCR products from NDV-infected MEFs sampled over a 72 h time course with [γ^{32}P]ATP-labelled-IP-α4. Panel b shows the results using pooled PCR products from MCMV-infected MEFs performed in the same bag as for samples in panel a. Panel d and panel e show the results of the hybridisation between PCR products from NDV-infected MEFs and MCMV-infected MEFs, respectively, and [γ^{32}P]ATP-labelled-IP-α1. Panel c and panel f show the results of hybridisation of known amounts (shown on the left of the panels) of specific MuIFNs-α DNA fragments (indicated on the top of the two panels) amplified using the set of primers specific for MuIFNs-α on plasmid clones of each subtype. These standards were included in the same hybridisation bags as the test blots. The time points used in the 72 h time course are indicated on the top of panels a and d and the PCR volumes used are indicated by the numbers on the left on panels a, b, d and e ($10^0 = 10$ μl and $10^{-1} = 1$ μl).

[γ-^{32}P]ATP and hybridised to the dot blotted PCR products. Fig. 2 (panels a and d) shows two typical sets of results from such a hybridisation reaction using [γ-^{32}P]ATP-labelled IP-α1 and IP-α4, respectively. Note that the target DNA blotted onto each membrane was identical for both panels. A control membrane dot blotted with known amounts of MuIFN-α subtype DNA (α1, α4, α5 and α6), produced from plasmid clones using the set of primers specific for all known MuIFNs-α, was included in each hybridisation reaction to ensure specificity of detection for each subtype. Fig. 2 (panels c and f) show the hybridisation of two such control membranes with [γ-^{32}P]ATP-labelled IP-α1 and IP-α4, respectively. As

shown in Fig. 2 (panels c and f), IP-α1 and IP-α4 were specific to MuIFN-α1 and α4 subtypes, respectively.

The signal intensities from hybridisation of the PCR products from each time point sampled were then compared to those of the different amounts of homologous DNA on the control membrane using laser densitometry. A score of 0 to 6, for signal intensity obtained from 'no DNA' control to that obtained when the signal intensity exceeds that for 50 ng of homologous DNA, was used. Based on this comparison, the relative levels of expression of MuIFN-αl, α4, α5 and α6 in NDV-infected MEFs could be determined assuming equivalent amplification of each subtype (see Section 4). All the data from the 4 strains of mice studied are summarised in Fig. 3 (panels a to d). Whilst comparison of MuIFN-α subtype levels was possible with this approach, the levels of MuIFN-β detected was only assessed from the intensity of ethidium bromide fluorescence of the 390 b.p. amplification product in 2% agarose gels, and this could not be directly related to the MuIFN-α subtype levels determined by hybridisation. The expression of MuIFN-β is thus denoted by an asterisk above the time points at which it was detected as shown in Fig. 3.

In NDV-infected MEFs from BALB/c mice, all the 4 MuIFN-α subtypes studied, as well as MuIFN-β, were expressed concurrently between 4 to 12 h p.i. Thereafter, only some of the 5 subtypes were expressed (Fig. 3, panel a). In contrast, NDV-infected MEFs from C57Bl/6 mice only expressed two MuIFN-α subtypes (α1 and α4 at 4 h p.i. and only α4 at 8 h p.i.) and MuIFN-β (between 4 to 10 h p.i.) over the same time course (Fig. 3, panel b). This strain specific difference in the pattern of type I IFNs expressed contrasts with the very similar kinetics and overall titers seen for these two strains (see previous section above and Fig. 1). Similarly, only 2

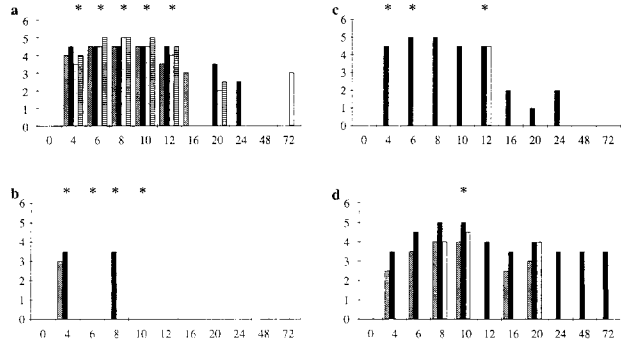

Fig. 3. Graphs showing the relative levels of expression of MuIFN-αl (▨), α4 (■), α5 (▩) and α6 (▤) in NDV-infected MEFs from BALB/c mice (panel a), C57Bl/6 mice (panel b), B6.C-*H-28*^{c} mice (panel c) and CBA mice (panel d). The relative levels of expression were determined by comparison of signal intensities, using laser densitometry of dot blotted PCR products with known quantities of control DNA hybridised with the [γ^{32}P]ATP-labelled oligonucleotides used for identification. The numbers on the vertical axis 0 to 6 refers to signal intensities observed when the following amounts of control MuIFN-α DNA were used: 0 = blank, 1 = 0.005 ng, 2 = 0.05 ng, 3 = 0.5 ng, 4 = 5 ng, 5 = 50 ng and 6 = > 50 ng. The time course over which the sampling was performed is given on the horizontal axis as h p.i. The expression of MuIFN-β over the same time course is represented by an asterisk above the panels.

MuIFN-α subtypes (α4 from 4 to 24 h p.i. and α5 at 12 h p.i.) and MuIFN-β (detected between 4 and 12 h p.i.) were expressed in NDV-infected MEFs from B6.C-*H-28*^{c} mice (Fig. 3, panel c). This B6.C-*H-28*^{c} strain differs from the C57Bl/6 strain by a BALB/c allele at the *If-1* locus (see Section 4). In NDV-infected MEFs from CBA mice, a different pattern of type 1 subtype expression was seen. MuIFN-α4 was expressed from 4 to 72 h p.i. and MuIFNs-αl and α5 were also expressed up to 20 h p.i., whilst MuIFN-β was only detected at 10 h p.i. (Fig. 3, panel d). In general, of the 5 type I IFN subtypes studied, MuIFN-α4 and β were the most commonly expressed subtypes, followed by α1, α5 and α6.

3.3. Total acid-stable type I IFN response in MCMV-infected MEFs

The total type I IFN present in the supernatants from control and MCMV-infected MEFs from the 4 strains of mice used were dialysed against glycine HCl (pH 2) for 2 days and against MOBS (pH 7.2) for another 2 days. The total acid-stable type I IFN was then titrated using the CPE reduction bioassay and the titers are presented in Fig. 4. For the MEFs derived from 3 out of the 4 strains of mice studied (BALB/c, C57Bl/6 and B6.C-*H-28*^{c}) the kinetics of total acid-stable type I IFN induction are essentially similar to those observed following NDV infection. However, it should be noted that the overall titres at any given time point are at least 5 to 10-fold lower than those following NDV infection (compare Figs. 1 and 4). In the case of MEFs from CBA mice, a lag phase of about 10 h before type I MuIFN titers could be detected in supernatants, was seen after MCMV infection. The titres, there-

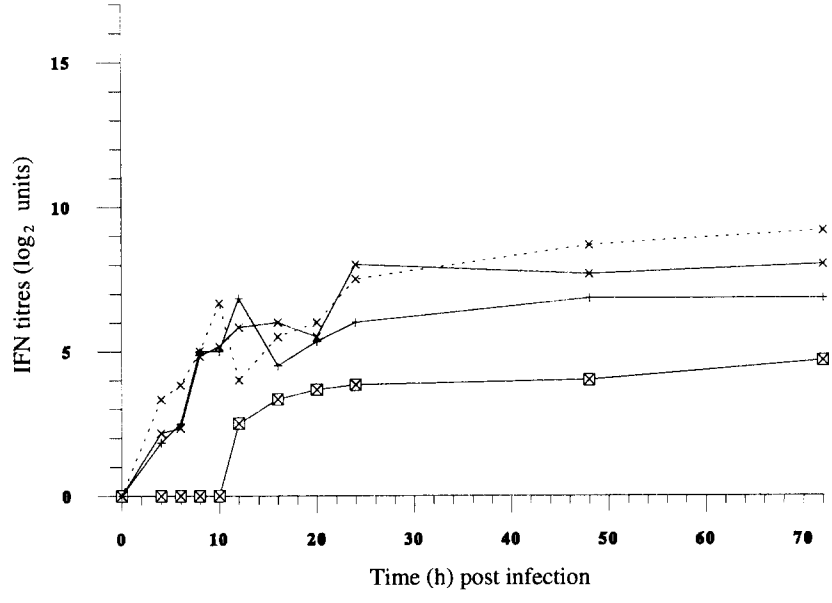

Fig. 4. Graph showing total acid-stable type I MuIFN titres in supernatants of MCMV-infected MEFs from BALB/c (—|—), C57Bl/6 (—×—) B6.C-*H-28*^{c} (--×--) and CBA (—⊠—) mice at various times (h) p.i.

after, were significantly lower than the titres of MCMV-infected MEFs from the three other strains throughout the whole time course.

3.4. Type I IFN analysis in MCMV-infected MEFs

After removal of supernatant from control and MCMV-infected MEFs for acid-stable type I IFN titration over the 72 h time course, the MEFs were washed in MOBS. Total cytoplasmic RNA was extracted and cDNA was synthesised and amplified as described earlier for NDV-infected MEFs. Agarose gel electrophoresis of PCR products only revealed the presence of the 308 b.p. and 390 b.p. fragments of MuIFNs-α and MuIFN-β, respectively, in MCMV-infected MEFs from BALB/c mice, but not from the 3 other strains of mice. The 308 b.p. fragment of MuIFNs-α was detected at 4, 6, 8, 10 and 12 h and the 390 b.p. fragment of MuIFN-β was detected at 10 h post-MCMV infection of MEFs from BALB/c mice (data not shown). The expression of MuIFN-β is presented as an asterisk above the time point at which it was detected (see Fig. 5, panel a).

Despite the absence of detectable bands on agarose gels in MCMV-infected MEFs from all the strains except BALB/c, dot blot analysis was conducted on the pooled PCR products from each time point. The preparation of the membranes, both test and control, was as described earlier for NDV infection. In fact, the hybridisation reactions for the samples were carried out in the same bags as those for NDV-infected MEFs, and the target DNA blotted onto the nitrocellulose membranes shown in panels b and e of Fig. 2 are identical. Using the method of comparison of signal intensities between test and control dot blotted samples described for NDV infection, the relative expression of the 4 MuIFN-α subtypes in MCMV-in-

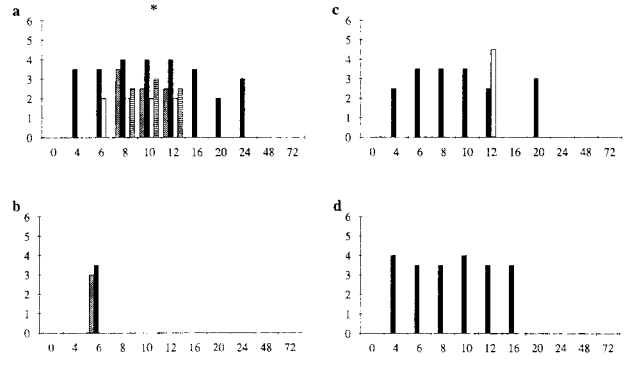

Fig. 5. Graphs showing the relative levels of expression of MuIFN-α1 (▨), α4 (■), α5 (▩) and α6 (▤) in MCMV-infected MEFs from BALB/c mice (panel a), C57Bl/6 mice (panel b), B6.C-*H-28*^{c} mice (panel c) and CBA mice (panel d). The relative levels of expression were determined by comparison of signal intensities, using laser densitometry, of dot blotted PCR products with known quantities of control DNA hybridised with the [γ^{32}P]ATP-labelled oligonucleotides used for identification. The numbers on the vertical axis 0 to 6 refers to signal intensities observed when the following amounts of control MuIFN-α DNA were used: 0 = blank, 1 = 0.005 ng, 2 = 0.05 ng, 3 = 0.5 ng, 4 = 5 ng, 5 = 50 ng and 6 = 50 ng. The time course over which the sampling was performed is given on the horizontal axis as h p.i. The expression of MuIFN-β over the same time course is represented by an asterisk above the panels.

fected MEFs was obtained. A summary of these relative levels is presented in Figs. 5 (panels a to d). Similar to the NDV infection, the MCMV infection of MEFs from BALB/c mice revealed that all the 5 type I MuIFN subtypes were expressed. As shown in Fig. 5 (panel a), between 8 to 12 h p.i. all the 4 MuIFN-α subtypes were expressed, and at 4 h and 16 to 24 h p.i. only MuIFN-α4 was expressed. In contrast to the MEFs from BALB/c mice, only MuIFN-α1 and α4 were expressed in MEFs from C57Bl/6 mice, and their expression was only detected at 6 h p.i. (Fig. 5, panel b). In the MEFs from B6.C-*H-28*^{c} and CBA mice, MuIFN-α4 was the predominant subtype expressed (Fig. 5, panels c and d). It is notable that in the CBA case, the detection of MuIFN-α4 message occurred 4 to 16 h p.i., despite the lack of a detectable titer until 12 h p.i. (see Section 4). In general, as was in the case for NDV-infected MEFs, MuIFN-α4 was the predominant subtype expressed in all the MCMV-infected MEFs inspite of MCMV being the weaker IFN inducer. The MuIFN-β subtype was not commonly seen.

Comparison of the data from NDV- and MCMV-infected MEFs shows that there is a difference in the total acid-stable type I IFN response. The titers from NDV-infected MEFs from all 4 strains of mice are at least 5- to 10-fold higher than those from MCMV-infected MEFs at every time point sampled. This difference in levels of expression is also reflected in the arbitrary signal intensity scales used in Figs. 3 and 5. In the case of the good inducer (NDV), signals often reached 5 units (Fig. 3), whilst in the case of the poor inducer (MCMV), signals exceed 4 units only in one instance (Fig. 5, panel c). Broadly speaking, the subtype expression patterns are similar for a given strain of MEFs, for example, BALB/c expressing the largest number of subtypes (Figs. 3a and 5a) irrespective of the virus used for the induction. Similar subtypes were expressed by MEFs from the same strain of mice as in the case of C57Bl/6 and B.6-C-*H-28*^{c} mice (compare Figs. 3b with 5b and 3c with 5c).

4. Discussion

In this study, one notable observation is the relationship between the pattern of type I IFN subtype expression and the genotype of the MEFs. The range of type I IFN subtypes expressed appears to be cell genotype dependent. Comparison of Fig. 3 (NDV infections) with Fig. 5 (MCMV infections) shows that in 3 out of the 4 strains of mice used (BALB/c, C57B1/6 and B6.C-*H-28*^{c}) the same MuIFN-α subtypes were expressed irrespective of the virus used. In the CBA case the predominant IFN-α4 patterns are very similar with both viruses, but NDV induction shows low levels of IFN-α1 and α5 that are not seen with MCMV infection. A further difference in the expression patterns is the expression of MuIFN-β, detected in all NDV-infected MEFs and only detected in MCMV-infected MEFs from BALB/c mice. It should, however, be pointed out that the expression of MuIFN-β in this study was assessed from ethidium bromide fluorescence of the 390 b.p. PCR product in 2% agarose gels. Hence, MuIFN-β detection only occurs if the concentration of the PCR products is relatively high (at least 20 ng). Thus, the MuIFN-β expression patterns may be less different if a more sensitive detection technique is used. This

was the case for IFN-α subtype detection where hybridisation to specific subtype oligonucleotides (IPs, see Table 1) was employed. Indeed, in most MCMV infections of MEFs, the PCR products were not detectable on electrophoretic analysis. Neverthelesss, whilst the sensitivity of the detection methodology and the dosage of infecting virus may influence the details of the expression patterns seen, the general feature is that the cell genotype is the major determinant of the subtype patterns seen.

The total acid-stable type I IFN response, unlike subtype expression patterns, appears to be inducer-dependent. The acid-stable type I IFN titers from NDV-infected MEFs were at least 5- to 10-fold higher than those from MCMV-infected MEFs (compare Fig. 1 with Fig. 4). This difference in titers is attributed to the nature of the virus used for induction as RNA viruses, in this case NDV, have been reported to be more efficient in inducing IFNs than DNA viruses (in this case the MCMV, DeMaeyer and DeMaeyer-Guignard, 1979). In both MCMV and NDV infections of MEFs, the overall final IFN titers (with the exception of those from MCMV-infected MEFs from CBA mice, see next paragraph), appear to be independent of the subtype expression patterns. For example, the the total acid-stable IFN titres from NDV-infected MEFs from BALB/c mice are the same as those from NDV-infected MEFs from the C57Bl/6 mice, whilst their subtype expression patterns are very different. In the BALB/c case, all four IFN-α subtypes examined in this study and IFN-β are expressed over an extensive part of the time course (Fig. 3, panel a). However, in the C57Bl/6 case only IFN-α4 and α1 mRNAs are expressed at early time points along with IFN-β (Fig. 3, panel b). The equivalence of the titers in both cases, despite the differences in subtype composition suggests a feedback mechanism. It would appear from these studies that the nature of the inducer sets the limit of the overall type I IFN titers seen, whilst the genotype of the cells determines the subtype mix that gives rise to that titre. The fact that a few subtypes can give the same titres as many suggests that some "cross-talk" between individual subtype genes with regard to the level of expression is occurring. This observation has a precedent in multigene families in eukaryotes. The isoforms of the various actins present in mice vary considerably with developmental age, yet an upper limit to the total amount is maintained. Loss of the cardiac form of actin due to disease leads to a compensatory increase in the level of skeletal actin (Garner et al., 1989). Direct experimental proof that such a mechanism may be operating with the type I IFN gene family is being pursued in our laboratory at present.

As mentioned in the preceeding paragraph, the only variation in the total acid-stable type I response was seen in the MCMV-infected MEFs from CBA mice. Here, there was a 10 h lag phase in the detection of IFN in the supernatant, but the type I IFN subtype mRNA expression was detected as early as 4 h p.i. as was seen in MEFs from all of the other strains of mice. This delay in detection of IFN in the supernatant of MCMV-infected MEFs from the CBA strain must be due to a delay in post-transcriptional events; either a delay in translation or a delay in secretion of the translated products. We have also observed that in the MCMV-infected MEFs from CBA mice, CPE was detected later, and the CPE was less severe as compared with MEFs from the other 3 strains (data not shown). It may be that these observa-

tions are of physiological significance as CBA mice are considered to be a relatively resistant strain to in vivo MCMV infection (Grundy et al., 1981). Further reports in the IFN literature of potential relevance to this particular point are observations by Jilbert et al. (1986) and Shuttleworth et al. (1983). Jilbert et al. (1986) reported on antibody detection of type I IFN in the cytoplasm of infiltrating white blood cells around foci of viral replication in the livers of Hepatitis B patients. Shuttleworth et al. (1983) reported the absence of IFN-β activity but presence of IFN-β mRNA in human lymphoblastoid cells infected with Sendai virus.

A final observation from the present study concerns the nature of the *If-1* locus. The *If-1* locus effects the serum IFN titres seen in mouse strains following in vivo NDV infection (DeMaeyer and DeMaeyer-Guignard, 1969). C57Bl/6 mice carry the *If-1*^{h} allele which determines the higher serum IFN titres seen in this strain. BALB/c mice carry the *If-1*^{l} allele which is associated with the 10-fold lower serum IFN titres of BALB/c mice. The congenic B6.C-*H-28*^{c} mice carry the *If-1*^{l} allele on a C57Bl/6 genetic background (Bailey, 1971). In recent years, the nature of the *If-1* locus, found to be expressed in the spleen and macrophages, has been the subject of investigation (Daigneault et al., 1988; Zawatsky and Homfeld, 1990; Zawatsky et al., 1991; Raj et al., 1992; Daigneault and Skup, 1992; Zawatsky and Wurmbaeck, 1992). In the spleens of NDV-infected C57Bl/6 (*If-1*^{h}) and BALB/c (*If-1*^{l}) mice, IFN-α1, α4 and α6 genes were expressed but their levels of expression in the C57Bl/6 mice were 10- to 20-fold higher. This indicated that the *If-1* locus affects the expression of all IFN-α subtypes and is not associated with the deletion or inactivation of a specific IFN gene (Raj et al., 1992). In the present study, the *If-1* locus was found not to affect the IFN levels produced by MEFs in vitro. This is consistent with previous studies showing that this locus is not operating in MEFs (DeMaeyer and DeMaeyer-Guignard, 1979). However a different observation has been made using MEFs in the present study. Examination of the type I IFN mRNAs expressed in the MEFs from each of these mouse strains (see panels a, b and c of Figs. 3 and 5) shows that the congenic B6.C-*H-28*^{c} patterns is most similar to that of the C57Bl/6 patterns. That is, a production of only IFN-α1 and α4 subtypes, a pattern quite distinct from the BALB/c production of all the subtypes examined. However, the presence of the BALB/c *If-1*^{l} allele, whilst not altering the C57Bl/6 subtype pattern, effected the time course or kinetics of the expression pattern. The prolonged (4 to 30 h) detection of expression is more characteristic of BALB/c cells than the shorter (4 to 8 h) mRNA expression period seen with C57Bl/6 cells. These observations suggest that the *If-1*^{l} allele is altering the kinetics of subtype expression. It should be noted that the overall titer has not been effected in any of these strains which agrees with previous reports that the *If-1* locus does not operate in embryonic cells to alter IFN levels produced.

We have been able to make some comments concerning the relative levels of expression of the four different IFN-α subtypes examined in this work. Such comparisons assume equal amplification, during the PCR steps of the procedures, of each IFN-α subtype. This assumption has been tested in two ways. Firstly, we used clones of each subtype in controlled experiments. Secondly, we analysed the relative IFN-α subtype expression levels in NDV-infected mouse L929 cells. The results were

in excellent agreement with previous data derived from independent Northern blotting and S1 protection experiments (Lai et al., 1994). We, therefore, feel this experimental approach will be useful for investigating further aspects of subtype expression, particularly in vivo. For example, whilst the present study and the previous data from this laboratory (Lai et al., 1994) has defined the influence of genotype on type I IFN subtype expression, only fibroblast cells have been studied. It may be that issue type will also influence the pattern of subtype expression. We are presently examining macrophages, liver cells, brain and muscle cells from individual strains in order to answer this question.

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