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# The in vivo expression patterns of individual type I interferon genes in murine cytomegalovirus infections.

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**Abstract**

Type I interferon (IFN) family has more than 10 structurally related subtypes of alpha IFN (*IFNA*) genes and a single beta IFN (*IFNB*) gene. This study examined the expression of *MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *MuIFNB* mRNAs in the livers and spleens of MCMV-infected mice at 2, 4 and 6 h post infection. The three strains of inbred mice studied were C57BL/6, B6.C-*H-28*$^c$ and BALB/c. B6.C-*H-28*$^c$ strain is a congenic strain of C57BL/6 genomic background. Detection of the specific mRNAs was by an established semiquantitative procedure based on reverse transcription and PCR amplification followed by differential hybridization to specific oligonucleotides. Similar expression patterns of the type I IFN mRNAs were found in C57BL/6 and B6.C-*H-28*$^c$ strains of mice. However, when the genotype was significantly different (BALB/c), a different expression pattern of IFN mRNAs was seen. Differences in the expression patterns of the type I IFN mRNAs was also seen between the livers and spleens of a given mouse strain. Thus, the present study indicates that mouse genotype appears to be a major determinant of the subtype response pattern seen in vivo and that tissue-type can influence the subtype response pattern seen within a given mouse genotype. © 1997 Elsevier Science B.V.

*Keywords:* Type I murine interferon response; Genotype; Tissue-type; Reverse transcription-polymerase chain reaction; In vivo

## 1. Introduction

Since 1957 when Isaacs and Lindenmann first reported the discovery of interferon, various aspects of the IFN system have been studied (Isaacs and Lindenmann, 1957). This has ranged from structure-function relationships to current interest on the cascading signal transduction and induction of IFN expression. For some years, it has been known that type I IFNs are a family which includes multiple copies of IFN alpha (IFN-$\alpha$)

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sub-species plus a single biologically active IFN beta (IFN-$\beta$) protein. Recently, IFN tau (IFN-$\tau$) and IFN omega (IFN-$\omega$) have also been added as members of the type I IFN family (Johnson et al., 1994). Nevertheless, it remains unknown as to why so many subtypes exists. Some researchers have hypothesised that the multiple IFN-$\alpha$ subspecies are a series of evolutionary duplicates of an original gene. It may therefore be the case that these subspecies are all of equivalent function in vivo or that each subtype has evolved its own special function (Finter, 1991). This question is of clinical importance if subtype function has differentiated.

In humans, the production of different human (Hu) IFN-$\alpha$ subtypes appears to be a carefully controlled process and is influenced by the method of interferon induction (Rubinstein, 1987). The cell type which is producing the interferon is also relevant. For example, monocytes and lymphocytes induced in the same manner will produce different mixtures of HuIFN-$\alpha$ subtypes (Goren et al., 1986). When various cells are induced to produce type I IFN there are usually at least 10 different IFN-$\alpha$ subtypes expressed.

The type I MuIFN family also consists of multiple MuIFN-$\alpha$ subspecies plus a single MuIFN-$\beta$ (Kelley and Pitha, 1985a; Zwarthoff et al., 1985; Dandoy et al., 1985). Hence this animal model is used to facilitate in depth studies on this multigene class. There have been several in vitro studies looking at the expression patterns of *MuIFNA* subtypes. When mouse L-cells were infected with Sendai virus, the expression of *MuIFNA4* and *B* genes predominated over *MuIFNA1*, *A2*, *A5* and *A6* (Zwarthoff et al., 1985). With Newcastle disease virus-infected macrophages, *MuIFNA1*, *A2*, *A4* and *B* were the dominant species produced (Hoss-Homfeld et al., 1989). Also, MuIFN-$\alpha$4 was the most highly expressed subtype with Newcastle disease virus infected mouse L cells. The MuIFN-$\alpha$4 subtype was expressed 5–15 fold higher than the other subtypes (Kelley and Pitha, 1985b). These observations were confirmed using the reverse transcription/polymerase chain reaction/identification (RT/PCR/ID) system developed in this laboratory and used in the present study (Lai et al., 1994a). Such studies on MuIFN-$\alpha$ subtype expression were conducted as in vitro experiments. The expression of these subtypes in vivo is not well characterized. Consequently, the present study has chosen a specific set of Mu type I genes and examined their expression in vivo following MCMV infection.

Early evidence demonstrated that MCMV is a poor inducer of IFN (Osborn and Medearis, 1966). However, IFN does play an important role in the early defence mechanism against MCMV infection. Grundy et al. (1982) reported an increase in the susceptibility of adult mice to MCMV following the administration of antiserum specific for type I IFN, and that the levels of IFN are controlled by non-*H-2*-linked genes. Subsequent studies have shown that type I IFNs play an important role in MCMV replication, and that mouse genotypes were associated with resistance to MCMV infection in that the lethal dose of MCMV for C57BL/6 mice is 2–4 fold higher than for BALB/c mice (Allan and Shellam, 1984, 1985; Quinnan and Manischewitz, 1987; Yamaguchi et al., 1988; Martinotti et al., 1990, 1992, 1993). Hence, this is a useful disease model to study the possible different expression levels of IFN-$\alpha$ subtypes across mouse strains and tissue types.

In light of the above points, the present study investigated the expression patterns of the *MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *B* subtypes in response to an MCMV challenge. *MuIFNA1*, *A4*, *A5* and *A6* were selected because they are clustered on a 28 kb fragment of mouse chromosome 4 (Kelley and Pitha, 1985a) and may hence be subject to a common local regulatory mechanism. *MuIFNA9* and *B* were selected as they do not form part of this genomic cluster where the other genes selected are located. A RT/PCR/ID system reported earlier by this laboratory (Lai et al., 1994a,b) was used to compare the early expression patterns of these MuIFN subtype mRNAs in the liver and spleen samples from mice at 2, 4 and 6 h post MCMV infection. The mice studied were the C57BL/6, BALB/c and B6.C-*H-28*$^c$ strains. B6.C-H-*28*$^c$ is a congenic strain known to carry two BALB/c loci (a minor histocomaptibility locus, *H-28*, 14 cM of intervening DNA and the *If-1* locus) on the C57BL/6 genomic background (De Maeyer and De Maeyer-Guignard, 1969,

1979). The *If-1* locus is virus specific and governs the circulating levels of IFNs following Newcastle disease virus (NDV) infection of mice and, hence, is not relevant to MCMV infection. The B6.C-*H-28*$^c$ strain is therefore essentially a second C57BL/6 genotype. The results show that the same or highly similar expression patterns of type I IFN mRNAs were found in C57BL/6 and B6.C-*H-28*$^c$ strains of mice. However, when the genotype was different (BALB/c) the expression pattern of type I IFN mRNAs seen was altered. The results also show that there are differences in the expression patterns of the type I IFN mRNAs when the livers and spleens within a given mouse strain are compared.

## 2. Materials and methods

### 2.1. Mice

Inbred specific pathogen free C57BL/6, BALB/c and B6.C-*H-28*$^c$ mice were obtained from the Animal Resources Centre (Murdoch, W.A., Australia) and kept under a specific pathogen-free environment. Groups of four C57BL/6, BALB/c and B6.C-H-*28*$^c$ female mice at 6–8 weeks of age were used for each time point.

### 2.2. Cell lines and virus stocks

L929 cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10% foetal bovine serum.

Murine cytomegalovirus (MCMV; Perth, K181) was propagated in the salivary gland of weanling, female BALB/c mice, and the virus stock was titrated with the methyl cellulose overlay method (Allan and Shellam, 1984). Encephalomyocarditis virus (EMCV) was propagated in L929 cells and the titer of EMCV was determined as 50% tissue culture infectious dose ($TCID_{50}$) in L929 cells.

### 2.3. IFN induction and sampling

All infected mice were given 100 $\mu$l intraperitoneal (i.p.) inoculation of MCMV at $10^{5.67}$ pfu/mouse; approximately four times the BALB/c $LD_{50}$, a known lethal dose for BALB/c and C57BL/6 strains (Allan and Shellam, 1984). The difference in plasma IFN titres between strains of mice at 6 h post infection is maximized by this dose of MCMV (Allan and Shellam, 1985). The non-infected mice were inoculated with 100 $\mu$l of the normal salivary gland homogenate.

The liver and spleen from each mouse was removed aseptically at 2, 4 and 6 h post inoculation (p.i.). Each liver was halved, and the liver-halves from each mouse within a group were pooled. One pool of liver-halves was homogenized for IFN titration and the other pool of liver-halves was used for the RT/PCR/ID analysis of type I IFNs. The spleen from each mouse was treated the same way. All tissue samples were snap-frozen in liquid nitrogen.

### 2.4. Total RNA isolation

Liver and spleen halves were homogenized under liquid nitrogen, and total RNAs were extracted from these homogenates with Ultraspec™ (BIOTECX Laboratories, Texas) according to the manufacturer's recommendations. The total cytoplasmic RNA preparations were DNAse-treated to remove any IFN genomic DNA (Lai et al., 1994a). This was a vital step because the presence of any contaminating IFN DNA would create false positive results as the genomic genes do not contain introns. The absence of contaminating IFN DNA in the DNAse-treated RNA preparation was confirmed by direct PCR with primers specific for the amplification of type I MuIFNs (Table 1). The quality of the total cytoplasmic RNA was checked by looking at the integrity of its 18S and 28S ribosomal RNA (rRNA) as electrophoresed through a formaldehyde–agarose gel (Sambrook et al., 1989). The quantity of the total cytoplasmic RNA preparation was also estimated on this gel, relative to 2 $\mu$g of standard rRNA electrophoresed on the same gel.

### 2.5. RT/PCR/ID analysis of type I IFNs

The RT/PCR/ID-based technique for the detection and identification of type I IFN subtypes has previously been described in detail (Lai et al.,

Table 1
Sequences, $T_m$ and location of each primer for the PCR[a] amplification of *MuIFNA* and *B*

| PCR primers | Sequences | $T_m$[b] | Location on the sequence[c] |
|---|---|---|---|
| *MuIFNA* primers | | | |
| Upstream | 5′-T CTC TCC TGC CTG AAG GAC-3′ | 60 | 78–96 |
| Downstream | 5′-A CAC AGT GAT CCT GTG GAA-3′ | 56 | 370–388 |
| *MuIFNB* primers | | | |
| Upstream | 5′-CAG CTC CAA GAA AGG ACG AA-3′ | 60 | 19–38 |
| Downstream | 5′-GTA GCT GTT GTA CTT CAT GAG-3′ | 60 | 388–408 |

[a] PCR conditions involved an initial cycle of denaturation at 95°C for 5 min, annealing at 55°C for 2 min and extension at 70°C for 4 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 70°C for 2 min.
[b] $T_m = 2(A+T)+4(G+C)$.
[c] Consensus nucleic acid sequence of the mature coding region of the gene.

1994a,b). Briefly, 1 $\mu$g of the DNAse-treated total cytoplasmic RNA was reverse-transcribed, with random hexamers as primers, to generate a cDNA pool from all mRNA present in the input RNA. A further check of RNA quantity was performed post-RT by amplifying a 348 base pairs (bp) fragment of the mouse $\beta$-actin complementary DNA (cDNA) by PCR as reported by Murray et al. (1990). The plasmid pMCQ, which was constructed by Platzer et al. (1992) to include the sense and antisense of mouse $\beta$-actin specific primer sequences, was used as a positive control for the mouse $\beta$-actin PCR. The levels of Mu$\beta$-actin mRNA was confirmed to be the same for all strains at all time points before the second stage of this detection system. This involved the amplification of a 308 bp fragment from all *MuIFNA* cDNA by PCR with *MuIFNA* specific primers corresponding to the conserved region of all sequenced *MuIFNA* genes (Table 1). A separate set of primers were made for the amplification of a 390 bp fragment of *MuIFNB* (Table 1). The final stage of the RT/PCR/ID system involved the identification of the amplified individual *MuIFNA* and *B* subtypes by differential hybridization of the PCR products to [$\gamma$-$^{32}$P]ATP-labelled oligonucleotides specific to the individual *MuIFNA* and *B* subtypes. These oligonucleotides are referred to as identifying primers (IP) and they are designated *IPIFNA1*, *IPIFNA4*, *IPIFNA5*, *IPIFNA6*, *IPIFNA9* and *IPIFNB*, specific for identifying the cDNA of *MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *B*, respectively. The lengths, melting temperatures ($T_m$) and locations of the identifying oligonucleotides are presented in Table 2. Ten-fold serial dilutions of amplified *MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *B* DNA clones, ranging from 0.005–50 ng, were dot blotted onto a Hybond $N^+$ (Amersham) membrane. This (control) membrane was always co-hybridized with the test membrane (dot-blotted with 10 and 1 $\mu$l of amplified DNAs) to ensure specificity and give some indication of the relative amounts of each subtype amplified. The signal intensities of the dots from the test membrane were compared to the signal intensities from the control membrane. The data was semi-quantitated as follows: $+++$ for hybridization signals stronger than 200 ng of standard; $++$ for hybridization signals stronger than the 50 ng standard; $+$ for hybridization signals stronger than the 5 ng standard; $-$ for hybridization signals weaker than the 5 ng standard.

### 2.6. IFN titration

Fifty percent homogenates of livers and 20% homogenates of spleens were prepared from the remaining halves of livers and spleens. The acid-stable total type I IFNs present in liver and spleen samples were selected (Lai et al., 1994b) and titrated by the cytopathic effect (CPE) reduction assay in L929 cells infected with EMCV (Shellam et al., 1981). The MuIFN-$\alpha/\beta$ mix from Lee Biomolecular (San Diego, CA) was used as the IFN standard in the CPE reduction bioassay.

Table 2
Sequences and location of the identifying probes (IP) specific for *MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *B* subtypes

| Designation for IP[a] | Sequences of oligonucleotides used as specific hybridizaiton probes for the identification of type I MuIFN subtypes | Location of the sequence[b] |
|---|---|---|
| *IPIFNA1* | 5′-A TTT CCC CTG ACC CAG GAA GAT G-3′ | 324–346 |
| *IPIFNA4* | 5′-CC TGT GTG ATG CAG GAA CCT CC-3′ | 293–329 |
| *IPIFNA5* | 5′-T GAA GTC CAT CAG CAG CTC AAT-3′ | 261–282 |
| *IPIFNA6* | 5′-CAG GTA GAG ATA CAG GCA CTT CC-3′ | 307–329 |
| *IPIFNA9* | 5′-G CTG GTC GGG ATG AAG GAA CTG-3′ | 306–327 |
| *IPIFNB* | 5′-CGT CTC CTG GAT GAA CTC CAC C-3′ | 244–265 |

[a] The hybridization temperature used for *IPIFNA1*, *IPIFNA4*, *IPIFNA5*, *IPIFNA6* and *IPIFNB* was 65°C and that used for *IPIFNA9* was 60°C.
[b] Consensus nucleic acid sequence of the mature coding region of the gene.

## 3. Results

### 3.1. The acid-stable total type I IFNs in the livers and spleens of MCMV infected mice

Acid-stable total type I IFNs present in the pooled spleen halves from non-infected and infected mice were titrated by CPE reduction bioassay. IFNs were not detected in any of the uninfected spleen homogenates (data not shown). However, acid-stable type I IFNs were produced by MCMV-infected spleens from all three strains of mice over the 6 h time course (Fig. 1). The kinetics of IFN production in the spleens varied between strains of mice. Type I IFNs were detected in B6.C-*H-28*$^c$ (carriers of the BALB/c *If-1*$^l$ allele on C57BL/6 background) spleens after 2 h of induction and there was a lag of 2 h before the detection of type I IFNs in C57BL/6 and BALB/c spleens. The levels of IFN titrated from the spleens also varied between strains of mice. By 6 h the IFN titer from C57BL/6 spleens was about 8–16 fold (3–4 $\log_2$ Units) lower than the IFN titers from BALB/c and B6.C-*H-28*$^c$ spleens respectively (Fig. 1).

Acid-stable type I IFNs were titrated from the pooled liver halves from MCMV-infected mice over the 6 h time course (Fig. 1). Uninfected liver homogenates showed no detectable IFN (data not shown). The kinetics of IFN production in the infected livers were different from that observed in the infected spleens. IFNs were detected in C57BL/6 livers by 2 h p.i. However, there was a lag period of 4 and 6 h before IFNs were detected in the livers of B6.C-*H-28*$^c$ and BALB/c mice, respectively. Likewise, the levels of IFN varied between strains of mice. In contrast to the observed IFN titers in the spleens, by 6 h the IFN titer from C57BL/6 livers was about 32 fold (5 $\log_2$ Units) higher than the IFN titers from BALB/c and B6.C-*H-28*$^c$ livers (Fig. 1).

A comparison of the total IFN titers revealed differences in the IFN levels between livers and spleens from the same genotype of infected mice. After 6 h of induction, the IFN levels in C57BL/6 livers were 8 fold (3 $\log_2$ Units) higher than in C57BL/6 spleens. In contrast, at 6 h the IFN levels in the spleens of BALB/c and B6.C-*H-28*$^c$ mice were around 32 fold (5 $\log_2$ Units) higher than the IFN levels in the livers of the same strain.

### 3.2. Genotype-dependent differential expression of MuIFNA/B mRNA in spleens

The expression patterns of the six type I MuIFN subtype mRNAs from the spleens of MCMV-infected C57BL/6, B6.C-*H-28*$^c$ and BALB/c mice at 2, 4 and 6 h p.i. were compared using the RT/PCR/ID approach detailed in Section 2.5. The results of this analysis are shown in Table 3. The same set of MuIFN subtype mRNAs (*MuIFNA1*, *A4*, *A5*, *A9* and *B*) were identified from MCMV-infected C57BL/6 and B6.C-*H-28*$^c$ spleens at all time points. Notably *MuIFNA6* was not detected at any time point in the two C57BL/

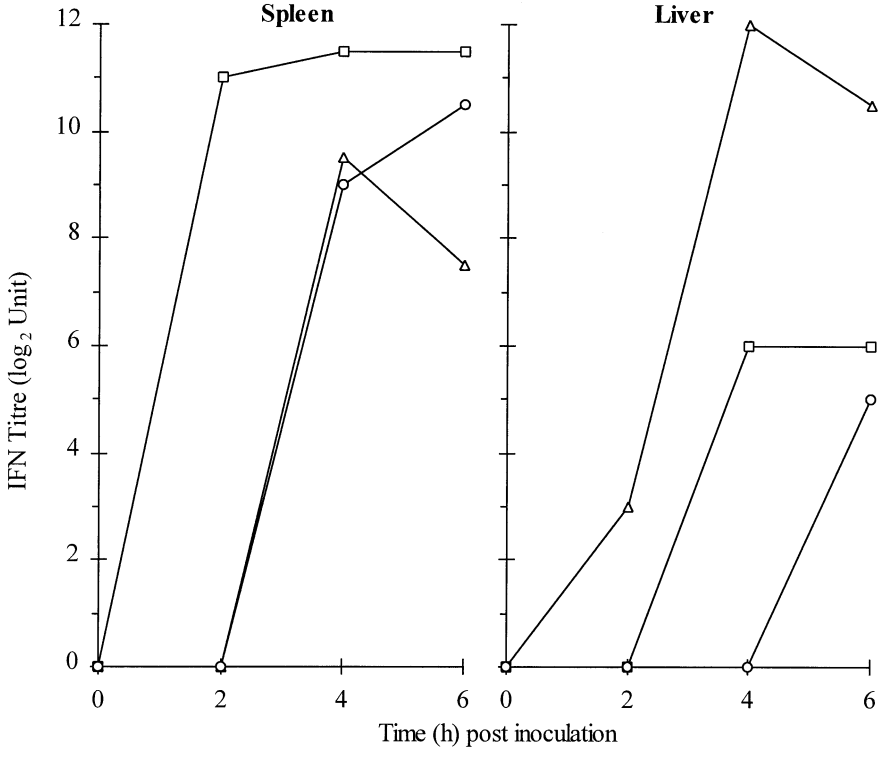


Fig. 1. The acid-stable total type I IFN titers in the livers and spleens of C57BL/6 (△), B6.C-*H-28*$^c$ (□) and BALB/c (○) mice. Homogenates were prepared from tissues pooled from four mice for each time point. The IFN titers were adjusted to 100% from the titer obtained from 50% homogenates of livers and 20% homogenates of spleens. Each mouse was inoculated with $10^{5.67}$ pfu of MCMV via the intraperitoneal route. 1 $\log_2$ unit (U) is approximately 3.9 IU/ml of the MuIFN-$\alpha/\beta$ standard (Lee Biomolecular, San Diego, California).

6 background strains. In BALB/c spleens the same set of IFN mRNAs was also detected; however, the *MuIFNA6* species was consistently and strongly present throughout the time course.

A further observation from the present study can be made by comparing the kinetics of IFN mRNA (Table 3) and IFN protein (Fig. 1) induction in the in vivo infected spleens. As early as after 2 h of MCMV infection, high levels of *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes were identified in C57BL/6 spleens and maintained throughout the time course, but IFNs were not detected in these tissues until after 4 h of MCMV infection. Similarly high levels of *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes, as well as *MuIFNA6*, were identified in BALB/c spleens after 2 h of MCMV infection and maintained throughout the time course, but IFNs were not detected in these tissues until after 4 h of MCMV infection. This lag in translation or secretion following transcription was also observed in the livers. However, the kinetics of IFN mRNA in B6.C-*H-28*$^c$ spleens correlated with the kinetics of the titrated IFNs from these tissues. High levels of *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes were identified and high levels of IFNs were titrated in B6.C-*H-28*$^c$ spleens after 2 h of MCMV infection and maintained throughout the time course.

### 3.3. Genotype-dependent differential expression of MuIFNA/B mRNA in livers

The same set of type I IFN mRNAs present in the liver samples were examined by the RT/PCR/

Table 3
The type I MuIFN mRNA present[a] in MCMV-infected spleens from C57BL/6, B6.C-*H-28*$^c$ and BALB/c mice at 2, 4 and 6 h p.i[b]

| Time (h) p.i. | Type I muIFN subtypes | C57BL/6 | B6.C-*H-28*$^c$ | BALB/c |
|---|---|---|---|---|
| 2 | *MuIFNA1* | +++ | ++ | +++ |
| | *MuIFNA4* | +++ | ++ | +++ |
| | *MuIFNA5* | +++ | +++ | +++ |
| | *MuIFNA6* | − | − | +++ |
| | *MuIFNA9* | +++ | +++ | +++ |
| | *MuIFNB* | ++ | + | ++ |
| 4 | *MuIFNA1* | +++ | ++ | +++ |
| | *MuIFNA4* | +++ | ++ | +++ |
| | *MuIFNA5* | +++ | +++ | +++ |
| | *MuIFNA6* | − | − | +++ |
| | *MuIFNA9* | +++ | +++ | +++ |
| | *MuIFNB* | ++ | ++ | ++ |
| 6 | *MuIFNA1* | ++ | + | ++ |
| | *MuIFNA4* | +++ | +++ | +++ |
| | *MuIFNA5* | +++ | +++ | +++ |
| | *MuIFNA6* | − | − | ++ |
| | *MuIFNA9* | +++ | +++ | +++ |
| | *MuIFNB* | ++ | ++ | +++ |

[a] Hybridization intensities from the dot-blotted (10 and 1 $\mu$l of amplified DNA) membranes of spleen samples were compared to the hybridization intensities from the control membranes dot-blotted with 50, 5, 0.5, 0.05 and 0.005 ng of each MuIFN subtype (*A1*, *A4*, *A5*, *A6*, *A9* and *B*). Key: +++, >200 ng; ++, >50 ng; +, >5 ng; −, ≤5 ng.
[b] Similar type I MuIFN subtypes were found in MCMV-infected spleens of C57BL/6 and the congenic strain (B6.C-*H-28*$^c$; carriers of the BALB/c *If-1*$^l$ allele on C57BL/6 background), in comparison to the MuIFN expression patterns in BALB/c.

ID method as described above for MCMV-infected spleens. The expression patterns of these subtypes from the livers of MCMV-infected C57BL/6, B6.C-*H-28*$^c$ and BALB/c mice after 2, 4 and 6 h of induction are presented in Table 4. C57BL/6 livers showed the presence of mRNAs of *MuIFNA1*, *A4*, *A5* and *B* at all time points, whilst the congenic B6.C-*H-28*$^c$ strain showed the same set minus the *IFNB* signal. The BALB/c liver samples showed the full set of subtypes examined for each time point as was the case with BALB/c spleen samples.

There is again a lack in correlation between the kinetics of IFN mRNA expression and the kinetics of IFN production as measured by bioassay. For example in BALB/c livers, acid-stable type I IFNs were not detectable in these homogenates until after 6 h of MCMV induction (Fig. 1). In contrast, strong expression levels of the dominant subtypes (*MuIFNA4*, *A5*, *A6* and *B*) were detected as early as 2 h p.i. (Table 4).

Interestingly, the general levels of IFN mRNAs observed in the livers of all three strains are less than the levels of IFN mRNAs found in the corresponding three strains of spleens. This difference in mRNA levels between livers and spleens is reflected in the IFN titers for B6.C-*H-28*$^c$ and BALB/c but not C57BL/6 strains.

### 3.4. Tissue-type influence on the differential expression of MuIFNA/B mRNA

A definite tissue-type influence was observed by comparing the expression patterns of *MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *B* subtypes from livers and spleens within the same genotype. For example C57BL/6 spleens, *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes were identified whereas in C57BL/6 livers *MuIFNA1*, *A4*, *A5* and *B* subtypes were identified. In the congenic strain, *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes were identified in spleens but only *MuIFNA1*, *A4* and *A5* subtypes were identified in livers. Interestingly all six (*MuIFNA1*, *A4*,

Table 4
The type I MuIFN mRNA present[a] in MCMV-infected livers from C57BL/6, B6.C-*H-28*$^c$ and BALB/c mice at 2, 4 and 6 h p.i[b]

| Time (h) p.i. | Type I MuIFN subtypes | C57BL/6 | B6.C-*H-28*$^c$ | BALB/c |
|---|---|---|---|---|
| 2 | *MuIFNA1* | + | + | + |
| | *MuIFNA4* | + | + | ++ |
| | *MuIFNA5* | ++ | + | +++ |
| | *MuIFNA6* | − | − | ++ |
| | *MuIFNA9* | − | − | + |
| | *MuIFNB* | + | − | + |
| 4 | *MuIFNA1* | + | + | + |
| | *MuIFNA4* | ++ | ++ | ++ |
| | *MuIFNA5* | ++ | + | +++ |
| | *MuIFNA6* | − | − | ++ |
| | *MuIFNA9* | − | − | + |
| | *MuIFNB* | + | − | +++ |
| 6 | *MuIFNA1* | + | + | + |
| | *MuIFNA4* | ++ | ++ | ++ |
| | *MuIFNA5* | ++ | + | +++ |
| | *MuIFNA6* | − | − | ++ |
| | *MuIFNA9* | − | − | + |
| | *MuIFNB* | ++ | − | +++ |

[a] Hybridization intensities from the dot-blotted (10 and 1 $\mu$l of amplified DNA) membranes of liver samples were compared to the hybridization intensities from the control membranes dot-blotted with 50, 5, 0.5, 0.05 and 0.005 ng of each MuIFN subtype (*A1*, *A4*, *A5*, *A6*, *A9* and *B*). Key: +++, >200 ng; ++, >50ng; +, >5 ng; −, ≤5 ng.
[b] Similar type I MuIFN subtypes were found in MCMV-infected livers of C57BL/6 and B6.C-*H-28*$^c$ in comparison to the MuIFN expression patterns in BALB/c.

*A5*, *A6*, *A9* and *B*) subtypes were identified from the spleens and livers of BALB/c mice, however, the overall levels of these subtypes in the spleens are higher (compare Tables 3 and 4).

## 4. Discussion

A notable observation of this study is the relationship between the expression patterns of type I IFN subtypes and the genotype of the mice. The range of type I IFN subtypes expressed in vivo appears to be genotype-dependent. In C57BL/6 spleens *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes were identified throughout the time course. The same subtypes were also identified in the spleens of the congenic strain B6.C-*H-28*$^c$ (which only differs from the C57BL/6 genotype by the presence of the BALB/c *H-28* and *If-1* loci; De Maeyer and De Maeyer-Guignard, 1969, 1979). However in the livers, even the small genotype difference has affected the IFN expression pattern. In C57BL/6 livers, *MuIFNA6* and *A9* subtypes were missing, while in B6.C-*H-28*$^c$ livers *MuIFNA6*, *A9* and *B* subtypes were not detected. In BALB/c mice, all six subtypes were found in both livers and spleens (Table 3 and 4). Genotype influence on the expression patterns of IFNs have also been observed in several in vitro studies in our laboratory (Lai et al., 1994b; Yeow et al., 1997). We previously identified *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes in C57BL/6 and B6.C-*H-28*$^c$ splenocytes after 7 h of NDV infection, whereas *MuIFNA1*, *A4*, *A5*, *A6* and *A9* subtypes were identified in BALB/c splenocytes (Yeow et al., 1997). In that same study, *MuIFNA4* and *B* subtypes were detected in both C57BL/6 and B6.C-*H-28*$^c$ mouse embryo fibroblasts (MEFs) after 7 h of NDV infection, whereas *MuIFNA1*, *A4*, *A5*, *A6* and *B* subtypes were identified in BALB/c MEFs. We have also reported the influence of genotype on the IFN subtype expression in MEFs infected with either MCMV or NDV in vitro (Lai et al., 1994b).

The other observation from this study is the tissue-type influence on the expression patterns of the six (*MuIFNA1*, *A4*, *A5*, *A6*, *A9* and *B*) subtypes within the same genotype. For example, only *MuIFNA6* was not expressed in C57BL/6 spleens, whereas in C57BL/6 livers two subtypes (*MuIFNA6* and *A9*) were not expressed. Tissue-type influence is also observed in B6.C-*H-28*$^c$ mice, such that in these spleens only *MuIFNA6* was not expressed, whereas *MuIFNA6*, *A9* and *B* subtypes were not expressed in the congenic livers. Tissue-type influence on the expression patterns have also been reported from in vitro studies by us (Yeow et al., 1997) and other groups (Hiscott et al., 1984; Goren et al., 1986; Greenway et al., 1992). We have observed different IFN expression patterns between splenocytes and MEFs infected with NDV (Yeow et al., 1997). For example, *MuIFNA1*, *A4*, *A5*, *A9* and *B* subtypes were identified from splenocytes after 7 h of NDV infection, whereas only *MuIFNA4* and *B* subtypes were found in MEFs that were infected the same way.

Finally, a possible interpretation from our results may be that a subtype pattern in a given strain could be a determinant of that strain's susceptibility to MCMV infection. If this was the case, then one would expect to find similar type I IFN subtype patterns in the more resistant mice, which would be different from the more susceptible strains of mice. Recently, some preliminary data have been obtained regarding the type I IFN subtype patterns observed in CBA mice (a MCMV resistant strain). This strain has shown a similar pattern to that observed in the C57BL/6 strain of mice, and distinct from the BALB/c pattern (W.-S. Yeow and M. W. Beilharz, unpublished). Further studies regarding this issue are currently underway in our laboratory.

We had chosen *MuIFNA1*, *A4*, *A5* and *A6* because they are clustered on a 28 kb fragment (Kelley and Pitha, 1985a), and may perhaps be subjected to a common regulatory mechanism. However, we found in the present study that of the cluster of four IFN genes only *MuIFNA6* was not expressed in either C57BL/6 or the congenic strain. It would appear that these genes may be differentially regulated. Bisat et al. (1988) have reported differences in the transcriptional inducibility of *MuIFNA1* and *A4* promoter regions which they correlated to the relative levels of these two subtypes.

There are limitations to the conclusions that can be drawn from the present study. Firstly, more subtypes would have to be investigated for a complete description of the range of type I IFNs expressed in response to a viral challenge. Secondly, more genotypes would have to be investigated to strengthen the conclusion of genotypic influence on the IFN subtype expression patterns. For this point, we have recently obtained some preliminary data on CBA mice (which are MCMV resistant) which also shows the same strain-dependent IFN subtype pattern variation that is observed with C57BL/6, B6.C-*H-28*$^c$ and BALB/c mice (W.-S. Yeow and M. W. Beilharz, unpublished). Thirdly, a greater range of tissue-types would have to be investigated to confirm the tissue-type influence observed in the present study. We are currently addressing these points in our laboratory investigations.

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