not show interatomic S--O distances shorter than the sum of the Van der Waals radii, so that the low value of the Raman frequency has to be attributed only to the effect of the crystalline environment.

Table 1. SH frequency of thiols in the solid state

Thiol	SH (cm^{-1})
L-cysteine	2545
L-cysteine HCl	2570
L-cysteine-methylester	2565
Dithiothreitol	2570 (unresolved doublet)
	2582
	2588
Glutathione	2530

Table 2. SH frequency of thiols in solution

Thiol	Solvent*	SH (cm^{-1})
CH_3SH	CCl_4	2586
C_2H_5SH	CCl_4	2578
$C_6H_5CH_2SH$	CCl_4	2580
$C_2H_5O(CH_2)_2SH$	CCl_4	2585
$C_2H_5COOCH_2SH$	CCl_4	2582
$NH_2(CH_2)_2SH$	H_2O	2578
$SH-CH_2-(CHOH)_2CH_2SH$	H_2O	2588
$COOHCH_2SH$	H_2O	2580
$COOH-CH(NH_2)CH_2SH$	H_2O	2583
GSH	H_2O	2584

* Data in CCl_4 from Mori[8], data in H_2O this work.

On the other hand, in aqueous solution, the sulphydryl stretching vibration of GSH occurs at higher wavenumbers, 2584 cm^{-1}, and it is independent from pH. This frequency compares well with those of simple thiols, both in water and in CCl_4[8], as shown in table 2. The substantial identity of ν(SH) frequency for very different molecules in both polar and non-polar solvents (at concentration below 0.5 M) excludes a large occurrence of either intra- or intermolecular (solute-solute and solute solvent) hydrogen bonding. Moreover, in all thiols examined, the shape of the ν(SH) Raman band is very similar: it is an essentially symmetric band with a small tail at low frequency side and a typical width of about 25 cm^{-1} in water at room temperature.

All these facts clearly suggest that in GSH the sulphydryl group is free and its reactive behaviour is not influenced by specific H-bonding, but -more probably - by the state of nearby reactive groups.

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Mechanisms of heterotypic immunity against canine distemper[1]

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Summary. Hep-2 cells infected with measles virus (MV) for as short as 6 h became refractory to superinfection with canine distemper virus (CDV) but not to vesicular stomatitis virus (VSV). The exact mechanism of such interference is unknown but probably occurs after virus attachment and penetration. These results verify the suggestion that virus interference may be a mechanism of heterotypic protection against canine distemper.

The application of heterotypic vaccination to canine medicine has been exemplified by the use of measles virus (MV) for vaccination against canine distemper (CDV). Even though CDV antibodies do not neutralize the MV, other cross-reacting antigens of MV allows pups to be sensitized by MV in the presence of anti-CDV maternal antibody[2,3]. Although the principle of this heterotypic immunity remains obscure, several mechanisms have been proposed to account for this phenomenon. These include an induction of a cross reacting antibody response[4,5], delayed type hypersensitivity or cell mediated immunity[6], heterologous viral interference[7] and interferon[7]. Thus far, none of these suggestions has been verified. Supposedly different mechanisms occur at different times post-exposure to measles virus. Thus, humoral and cellular immune response would probably function at times later than 1 week post-vaccination whereas interference would occur earlier. Recently, we have shown that MV can replicate in both canine lymphocytes and macrophages[8] suggesting that blockage at the leukocyte level is possible and could be effective against CDV infection. The present report provides further evidence that MV can interfere with CDV and that interference may be the mayor mechanism of heterotypic immunity against canine distemper at least at early stages of infection.

Materials and methods. Hep-2 cells were grown in 60 mm tissue culture plates (Corning No.25010) in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). Confluent cultures were infected at a multiplicity of infection (MOI) of 1 with MV (Edmonston) or UV-inactivated MV (UVMV). Following a 1-h adsorption period the unadsorbed virus was removed and fresh MEM was added. At different times post-infection (0 h or 6 h) the cultures were superinfected with various concentrations of CDV. The number of CDV plaques were evaluated 24-36 h post-infection[9] and the amount of infectious CDV or MV released into the culture fluid 72-96 h later was determined by a plaque assay[9] on Hep-2 and Vero cells for CDV and MV respectively. To prevent interference by CDV when MV titres were being conducted, anti-CDV antibody was included in the plaque assay. Controls included Hep-2 cells infected with MV followed by vesicular stomatitis (VSV) to ensure that there was no nonspecific interference due to interferon.

To determine whether interference was due to interferon, the supernatant fluid from MV infected cultures were

harvested and titrated in a standard plaque reduction assay as described previously[10].

Radioactive-labeled CDV was prepared by propagating the virus in Vero cells int the presence of ^{3}[H]-amino acids and ^{3}[H]-uridine (1 μCi/ml, Amersham-Searle, Oakville, Ont.). The ^{3}[H]-labeled virus was first concentrated by ultracentrifugation at 37,000 × g for 90 min then purified on a 10–50% linear sucrose gradient by centrifugation at 56,000 × g for 60 min. This procedure yielded a preparation which was concentrated 50-fold in infectivity and 85% of the radioactivity could be specifically precipitated by 1 indirect immunoprecipitation test. A suspension of Hep-2 cells, in plastic culture tubes (1×10^5 cells per tube in 0.1 ml MEM) were incubated with equal volumes of either CDV, live or UV inactivated MV for 4 h at 4 °C washed 2 times in MEM (4 °C) and then further incubated with ^{3}[H]-labeled CDV under the same conditions. Normal Hep-2 cells incubated with ^{3}[H]-labeled CDV only were used as controls to measure the amount of virus adsorption in the absence of possible attachment interference. Following incubation for 4 h at 4 °C the cells were washed 3 times in MEM, the cells pelleted and the radioactivity was determined by a scintillation spectrophotometer.

Results and discussion. Even though both MV and CDV replicate in Hep-2 cells their characteristic cytopathology is distinct. Thus, CDV produced discrete plaques in 24–26 h, while MV produced generalized syncytia only after 48 h of infection[8]. Thus, this cell system allows easy differentiation and accurate quantitation of CDV plaques even in the presence of MV. Employing this system of differentiating CDV from MV we attempted to see whether prior infection of cells with measles resulted in a reduction of CDV replication. As summarized in the table, prior infection with either live or UV-irradiated MV dramatically reduced the level of CDV production even if CDV infection occurred immediately after MV infection. If infection with measles virus was allowed to occur for as little as 6 h the cells became totally refractory to superinfection with CDV and no infectious CDV was released. CDV could similarly interfere with replication and release of MV if CDV was used as the interfering virus. These results strongly support the hypothesis that CDV and MV can interfere with each other's replication and thus interference may play an important role in defense against CDV infections.

The observation that both live and UV-irradiated MV interfere with CDV, prompted us to extend our investigation to examine whether interference occurred at the cell receptor level[11] or whether it occured at the intracellular level in a manner analogous to defective particles[12]. To test whether interference occurred at the receptor level we first infected cells with UV-inactivated or noninactivated virus and then tested their ability to bind [^{3}H]-labeled CDV. Although there was a slightly lower level of [^{3}H]-CDV binding to cells previously exposed to MV this was not significant, indicating that CDV could still attach to cells previously infected with MV. This result was not unexpected since neither MV nor CDV possess neuraminidase[13] which could possibly cleave the receptor for the heterologous virus and thus prevent attachment. Furthermore, since the cell probably possesses in excess of 10^4 virus receptors/cell the input multiplicity (MOI of 1) would not be expected to saturate the receptors[14] and thus prevent attachment of the heterologous virus. These results further support the hypothesis that interference occurs at the translational or transcriptional stage of replication.

The possibility that interferon was involved in the observed interference phenomenon seems unlikely since interferon activity was not detected in the supernatant fluids of treated cells. Furthermore, interference was not observed with VSV, in the same system (results not shown), suggesting interferon was not involved.

Whether an accelerated antibody response[4,5] or cell mediated immunity[6] plays an important role in heterotypic immunity to distemper in vivo is presently a matter of speculation. Since the 2 viruses are antigenically related, it is possible that some cross reacting antigens are important in inducing immunity to the heterotypic virus. This type of immunity would, however, take at least a few days to develop and may therefore be effective only if animals were challenged at least 1 week after vaccination with MV. However, it appears that immediate protection (within 24 h) can occur following exposure to MV[15] suggesting that some defense mechanism(s) other than classical immune reactions are involved in preventing clinical disease[3,4,7]. Thus, it is logical to assume that viral interference at the cellular level may be the major mechanism of heterotypic immunity at the early stages post-vaccination. This is supported by the finding that MV can infect the same target cells (leukocytes)[8] that are normally infected with CDV. If this occurs, interference at the cellular level may also prevent the dramatic immunosuppression[16] generally observed with CDV. If immunosuppression is limited, the host's immune response can clear the virulent CDV and only a subclinical infection would occur. Therefore, it is postulated that interference is responsible for early protection, followed by the development of classical immunological defense mechanisms to cross-reacting antigens which provide protection at a later stage.

In vitro interference of measles virus (MV) and canine distemper virus (CDV) with each others replication

Treatment	Number of CDV plaques[a]	Virus yielded[b] CDV (PFU/ml × 10^3)	MV ($TCID_{50}$/ml × 10^4)
CDV	370 ± 86	41 ± 5.6	-
MV	-	-	13 ± 6
CDV-MV[c]	98 ± 23	12.4 ± 4.7	3.2 ± 1.4
MV-CDV[c]	60 ± 30	6.8 ± 3.5	6.4 ± 0.3
CDV-6h MV[d]	190 ± 43	38 ± 7.3	1.6 ± 0.8
MV-6h CDV[d]	0	0	1.0 ± 3.7
UVMV-6h CDV[d]	0	0	0

[a] Each culture was infected with an equal number of PFU (370) of CDV values represent the number of plaques observed in the specific cultures ± SD of triplicate cultures. [b] Values represent the virus yield from triplicate cultures ± SD. [c] Cultures were incubated for 1 h at 37 °C with the 1st virus in the combination, washed and immediately infected with the heterologous virus. [d] Cultures were incubated for 1 h at 37 °C with the 1st virus in the combination, washed and incubated a further 6 h in MEM ± 5% FBS before infection with the heterologous virus.

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