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Interferon Induction as a Quasispecies Marker of Vesicular Stomatitis Virus Populations†

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The interferon (IFN)-inducing capacity of different isolates of vesicular stomatitis virus (VSV) of the Indiana (IN) and New Jersey (NJ) serotypes were measured to assess the extent of variability of this phenotype. Over 200 preparations of wild-type field isolates, laboratory strains, and plaque-derived subpopulations were examined. Marked heterogeneity was found in the ability of these viruses to induce IFN, covering a 10,000-fold range. A good fit to a normal distribution for the log of the IFN yields suggests a continuum of incremental changes in the viral genome may govern the IFN-inducing capacity of consensus populations derived from independently arising infections. A broad range in the magnitude of these changes, skewed towards inducers of high IFN yields, is consistent with a comparable series of ribonucleotide changes in the VSV genome, a *sine qua non* of a quasispecies population. Plaque- or vesicle-derived populations displayed standard deviations less than the mean IFN yields, though skewed to higher yielders, whereas populations from field and laboratory samples which differed widely in time and origin of isolation gave standard deviations greater than the means. The plaque isolation of IFN-inducing particles of VSV-IN, normally masked in populations by the predominance of non-IFN-inducing particles that suppress IFN induction, and the isolation of potent wild-type IFN-inducing VSV-IN from cows during an outbreak of vesicular stomatitis in a region that had yielded only virus expressing the non-IFN-inducing phenotype in prior and subsequent years, supports the view that genetic bottlenecks are operative in the natural transmission of this disease.

The interferon (IFN)-inducing capacity of a virus is an attribute that can vary over a 10,000-fold range, even among closely related members of a family (30, 31, 43). In the case of vesicular stomatitis virus (VSV), its IFN-inducing capacity is regulated largely by the action of non-temperature-sensitive mutations (44). For VSV of the Indiana (IN) serotype, most isolates appear to regulate IFN induction in an all-or-none manner, i.e., they either induce IFN or they do not. VSV-IN isolates that induce little, or no, IFN express an antagonistic phenotype, namely, they suppress the induction of IFN in cells already programmed to induce it (20, 26, 27). In contrast, all VSV isolates of the New Jersey (NJ) serotype tested previously were good-to-excellent inducers of IFN that displayed a broad range of IFN-inducing capacities characteristic of each isolate (30). VSV-NJ appears to regulate the level of IFN induced by itself as well as any coinducing virus (14).

The maximum yield of IFN characteristically induced by a VSV population is thought to reflect a competition between the virus-initiated upregulation of the IFN gene(s) and its downregulation brought about by an as yet unidentified suppressor activity encoded by the virus, which is resident in its virion and extraordinarily active in chicken embryo cells (1, 4, 30, 31). Upregulation is thought to reflect the appearance in the cell of double-stranded (ds) RNA as the proximal inducer of IFN (21). This molecule then activates dsRNA-dependent protein kinase (18, 28), which leads to the subsequent activation of transcription factors and the IFN gene(s) (4, 17, 18, 49). VSV is thought to induce IFN gene activation in this manner in chicken embryo cell cultures (17).

The frequent outbreaks of vesicular stomatitis in cattle, horses, and swine caused by VSV have made numerous isolates of the virus available from widely diverse geographical and ecological regions (33, 38). The plethora of VSV isolates, the relatively simple viral genome (2), and the error-prone nature of its RNA replication (10) have led to the use of VSV as a model for phylogenetic studies and studies of molecular evolution (3, 7, 8, 16, 30, 32, 33, 38). The genomic variability of VSV is attributed to the high error rate of RNA virus polymerases with the consequence that quasispecies populations are generated with a vast repertoire of genomes upon which selection can play (5, 8, 9).

The term quasispecies as it relates to populations of RNA viruses has been used by Domingo and colleagues "... to represent a defined ensemble of related, nonidentical genomic sequences" (9). They point out that "... due to their error-prone replication, even clones of RNA genomes are not homogeneous collections of sequences, although they may behave as if they were" (9). The highly variable phenotype of IFN inducibility demonstrated for different field and laboratory isolates of vesicular stomatitis virus (VSV) (30), coupled with the ability to quantitate the IFN-inducing capacity of a virus population under conditions where the interaction of single, or a few, virions per cell is favored (22), permit a direct test of the quasispecies nature of VSV populations. These populations may also display an antagonistic phenotype, i.e., an IFN induction-suppressing capacity (20, 26, 27). The relative activities of these opposing phenotypes in quasispecies swarms could influence the intrinsic value of the IFN yield and hence might affect the course of a disease where the IFN system is involved.

This communication extends an earlier study (30) and compares the IFN-inducing capacities of a much larger and diverse number of VSV populations to understand ultimately the molecular basis for the regulation of a viral phenotype that is

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TABLE 1. VSV-IN and -NJ isolates ordered by IFN-inducing capacity

Serotype					
IN			NJ		
Laboratory code no. or strain	Isolate[a]	IFN yield ($U/10^7$ cells)	Laboratory code no. or strain	Isolate[a]	IFN yield ($U/10^7$ cells)
23	687CRB	50	62	986PNB	350
32	187ESB	50	63	1083PNB	520
24 (San Juan)	..NMB[b]	60	59	1283PNB	640
Orsay	..25INB	70	4	185PNB	680
26	..84GMB	80	14	..76ECM	700
Cocal	961TRG	80	6	1183CAB	800
22	287CRB	80	56	789CPB	850
Alagoas	..86CLL	80	60	185PNB2	920
35	..59PNL	90	61	785PNB	1,100
5	185GMB	90	55	..66TXB	1,170
27	986GMB	120	10	785NCB	1,500
30	1186ESB	180	69	589GAL	1,510
1	884PNB	180	17	..60PNB	1,700
8	..87VCB	180	71	1290CRB	1,770
31	1286ESB	180	Ogden	..49UTB1	1,800
15	..69PNL	200	49	185CRB	2,400
Mass.	..56NMB[b]	220	54	589VCB	2,400
34	183HDB	240	57	789CPB2	2,750
2	982HDB	240	Hazelhurst	..GAP	2,800
Toronto-HR	Unknown[b]	260	9	1084GMP	3,100
MS	Unknown[b]	280	50	785CRB	3,600
Glasgow	..42COE	300	58	789CPB3	3,900
13	284PNH	300	18	982HDB	4,000
16	..87OAB	320	3	1184HDB1	4,100
28	785ESB	360	47	1282CRB2	4,500
33	1183HDB	360	48	1284CRB	4,500
25	..86DFP	470	12	784OAB	4,500
29	.885ESB	690	64	986CRB2	5,300
19	784PNB	1,450	53	986CRB3	6,000
20	385CRB	1,900	7	1082CRB	6,100
21	385CRB	2,460	66	783GAP	6,400
11	284CRB	2,810	65	687CRB	11,300
			52	786CRB	12,000
			51	886CRB	45,000

[a] Isolates are ordered according to their capacity to induce IFN. Numbers indicate month, followed by year of clinical case. The dots indicate that the month is unknown. The abbreviations for geographical origins are CA, California; CL, Colombia; CO, Colorado; CR, Costa Rica; CP, Chiapas, Mexico; DF, Distrito Federal, Mexico; EC, Ecuador; ES, El Salvador; GA, Georgia; GM, Guatemala; HD, Honduras; IN, Indiana; NM, New Mexico; NC, Nicaragua; OA, Oaxaca, Mexico; PN, Panama; TR, Trinidad; TX, Texas; UT, Utah; and VC, Veracruz, Mexico. The abbreviations for species are B, bovine; E, equine; G, mites; H, human; L, sandflies; M, mosquitos; and P, porcine. A number after the species designation distinguishes different specimens from the same month and place.

[b] The source and history of several of the VSV isolates have been described previously (3, 30).

quantifiable over a 10,000-fold range. We show that the IFN-inducing capacity among closely related isolates of VSV can vary significantly, consistent with their quasispecies nature and the operation of genetic bottlenecks during natural infection.

MATERIALS AND METHODS

Cells and media. Monolayers of primary chicken embryo cells were prepared as previously described and aged in vitro for 7 to 11 days to enhance their IFN-inducing capacity (39, 42).

Source of viruses, preparation, and assay. The designation and origin of the wild-type field isolates and laboratory strains used in this study are displayed in Table 1. The nomenclature for the new isolates is based on that used earlier (30) and is described in footnote *a* of Table 1. All isolates were obtained and used with permission of the United States Department of Agriculture. Stocks of virus were grown in GMK-Vero cells infected at a low multiplicity of infection (10^{-4}) to obviate the production of defective interfering particles, including those intrinsically capable of inducing IFN (21, 23, 25). This means that about 1,000 cells were initially infected with a single infectious particle. In the context of this study each initial virus-cell encounter represented a genetic bottleneck, though the final population was a consensus of the dominant phenotype from multiply infected cells. Almost all field isolates tested represented first passage material grown in GMK-Vero cells. These cells do not respond to inducers of IFN and hence provide a neutral environment with respect to the IFN system (19, 41, 43). Some stocks of VSV were tested for the loss of IFN-inducing capacity after exposure to 50 lethal hits of 254-nm UV radiation (1 hit = 52.5 ergs/mm^2) (24) to confirm that this capacity was intrinsic to the standard particle and not the result of contaminating [±]RNA defective interfering particles capable of inducing IFN (23, 25). The IFN-inducing capacity of standard VSV is inactivated by this dose of UV radiation, whereas IFN induction by [±]RNA defective interfering particles is not (23, 25).

IFN induction and assay. Detailed protocols have been described for the generation and analysis of IFN induction dose-response curves (22) and for the assay of acid-stable type I IFN in primary chicken embryo cells (40, 43). Each series of assays included two standards as follows: (i) a stock of UV-irradiated avian reovirus (48), a highly reproducible inducer of IFN used to determine the intrinsic IFN-inducing capacity of the cells and to provide a reference standard with which to normalize the maximum yield of IFN between different batches of cells; and (ii) a laboratory reference standard of type I recombinant chicken IFN (41) included in each assay to determine the intrinsic capacity of each batch of chicken embryo cells to respond to the action of IFN. One laboratory unit of IFN was equivalent to 10 to 25 U of the MRC Reference Standard A 62/4 provided by the National Institute of Allergy and Infectious Diseases Research Resources Branch. Induction curves corresponded to the $r \geq 1$ model described previously (22). Therefore, the maximum yield of IFN for each virus could be calculated and validly compared.

When multiple assays of the IFN-inducing capacity of VSV isolates were compared, the standard deviation from the mean yield of IFN was ± 30%. For example, the IFN-inducing capacity of VSV-NJ (Hazelhurst) in nine different batches of primary chicken embryo cells was 2,300 ± 600 $U/10^7$ cells. Multiple determinations have shown that the relative IFN-inducing capacity between isolates is maintained, although absolute values may vary with the batch of primary chicken embryo cells. Thus, the VSV-IN isolate no. 22-20 in tens of assays always induces large amounts of IFN, while its sister plaque isolate no. 22-25 induces little or no IFN.

RESULTS

IFN-inducing capacity of field isolates of VSV-IN and VSV-NJ from outbreaks that differed widely in time and geographical location. Table 1 shows the IFN-inducing capacity of 32 isolates of VSV-IN along with the dates of their isolation, geographical locations, and host species. These isolates include six wild-type strains maintained in laboratories, Orsay (..25INB), Mass. (..56NMB), Toronto-HR (unknown), MS (unknown), Glasgow (..42COE), and San Juan (..NMB) (virus designations are in parentheses; see Table 1 for further explanation), and the related vesiculoviruses Cocal and Alagoas, arranged in ascending order of IFN yields. All but five isolates induced low yields of IFN in aged chicken embryo cells. Low yielders sometimes induced <10 $U/10^7$ cells (42, 44). Of the four best VSV-IN inducers of IFN, two were isolated in 1984, one in Alajuela province, Costa Rica (284CRB, Lab code no. 11), one in Chiriqui province, Panama (784PNB, no. 19), and two were isolated in 1985, from Cartago and Limon provinces in Costa Rica (385CRB, no. 20, and 385CRB2, no. 21). Tests of two other field isolates from Costa Rica (287CRB, no. 22) and (687CRB, no. 23), which were obtained in outbreaks 2 to 3 years later (in 1987) from geographically related areas in Alajuela province, showed they were weak inducers of IFN (Table 1; Fig. 1a). However, populations of isolate 287CRB (no. 22) harbored at least one IFN-inducing particle as a minor member of the population (see below). The histogram in Fig. 1a illustrates the IFN yields from these 32 isolates of VSV-IN ordered by their IFN-inducing capacity. These data reveal a 56-fold range of IFN yields, from 50 to 2,810 $U/10^7$ cells, with a standard deviation 1.5 times the mean yield (Table 2).

Table 1 also shows the IFN-inducing capacity and other characteristics of 34 field isolates of VSV-NJ. These include two laboratory strains, Ogden (..49UTB) and Hazelhurst

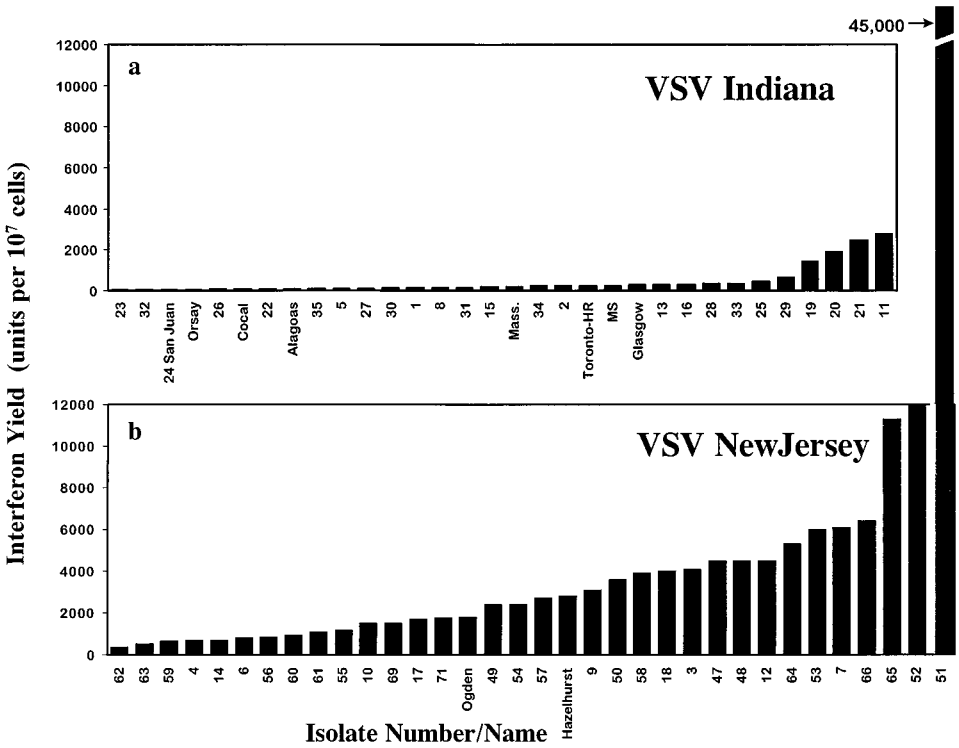

FIG. 1. Histograms which display in an ascending order the maximum yields of IFN induced in aged primary chicken embryo cells by the series of VSV IN (a) and NJ (b) isolates used in this study and described in Table 1. The procedure used to obtain the values recorded here through the generation and analysis of IFN induction dose (multiplicity)-response (IFN yield) curves has been described previously (24).

(..52GAP), with a long history of in vitro passages. In marked contrast to the VSV-IN field isolates (Table 1; Fig. 1a), all VSV-NJ isolates tested induced significant levels of IFN in aged primary chicken embryo cells. The histogram in Fig. 1b shows these 34 VSV-NJ isolates arranged in ascending order of IFN yields. These data reveal a 129-fold range of IFN yields, from 350 to 45,000 U/10^7 cells, and a standard deviation 1.7 times greater than the mean IFN yield (Table 2).

IFN-inducing capacity of VSV-IN plaque-derived populations from a single field isolate, 284CRB (no. 11). Most field isolates of VSV-IN induce little or no IFN (Table 1; Fig. 1a). However, a 1984 field isolate from an infected cow in Costa Rica was an exception (284CRB, no. 11). A stock of this virus induced 2,800 U of IFN per 10^7 cells. In order to determine the distribution of IFN-inducing capacities in this virus population, the virus stock was plaqued on GMK-Vero cells and 27 plaques were picked and amplified in one passage in GMK-Vero cells as described previously (44). All of the plaque-derived virus stocks produced $\geq 10^9$ plaque-forming particles (PFP)/ml. These subpopulations were used to generate IFN induction dose-response curves. Figure 2 is a histogram that displays as an ordered continuum the maximum yields of IFN induced by each plaque-derived stock from this field isolate. The IFN yields covered a 90-fold range from 160 to 14,300 U/10^7 cells, with a standard deviation 1.5 times less than the mean IFN yield (Table 2).

IFN-inducing capacity of a VSV-IN plaque-derived population from a non-IFN-inducing field isolate, 287CRB (no. 22). As previously demonstrated, most field isolates of VSV-IN induce little or no IFN and they concomitantly express a dominant phenotype, i.e., the suppression of IFN induction in a cell otherwise programmed to produce it (30). Under these conditions, a single particle suffices to suppress IFN production completely (26). Consequently, any IFN-inducing mutants that arise in populations that consist predominantly of non-IFN-inducing particles would be masked in multiply infected cells by the action of virions that express the IFN induction-suppressing phenotype. To circumvent this effect and make pos-

TABLE 2. IFN-inducing capacities of VSV field isolates and plaque-derived populations[a]

Virus type or designation	No. of samples	IFN yield (U/10^7 cells) (mean ± SD)	Range	Fold difference
VSV-IN isolates (first-passage populations)	32	451 ± 694	50–2810	56
VSV-NJ isolates (first-passage populations)	34	4,429 ± 7,688	350–45,000	129
VSV-IN no. 11 (plaque-derived subpopulations)	27	4,680 ± 3,560	160–14,310	89
VSV-NJ Lab no. 71 (vesicle plaque-derived subpopulations)	77	858 ± 404	310–2,790	9
VSV-IN Lab no. 22 (plaque-derived subpopulations)				
With no. 22-20	36	940 ± 5,165	15–31,000	2,100
Without no. 22-20	35	79 ± 33	15–168	11

[a] Results for IN isolates, NJ isolates, and subpopulations of no. 11, 71, and 22 are based on data used to generate Fig. 1a, 1b, 2, 4, and 3, respectively.

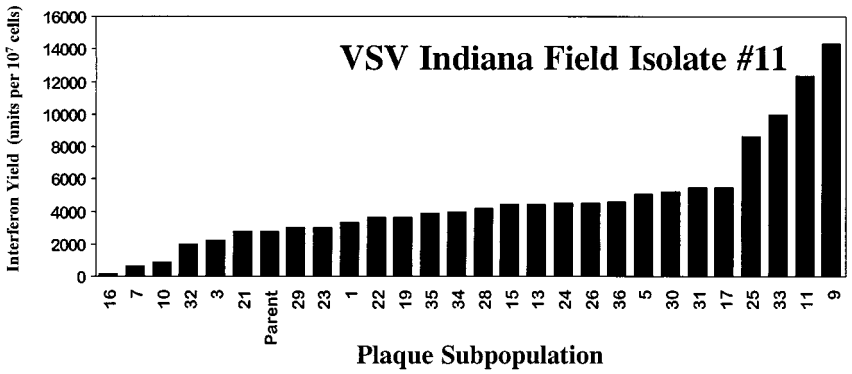

FIG. 2. Histogram which displays in an ascending order the maximum IFN yields generated in aged primary chicken embryo cell monolayers infected with 27 plaque-derived subpopulations of field isolate VSV-IN no. 11 (284CRB), along with that of the original parent.

sible the isolation and identification of individual members of the virus stock capable of inducing IFN, plaque-derived subpopulations were generated from the parent stock. Each subpopulation was tested for its IFN-inducing capacity. The histogram in Fig. 3 shows the IFN-inducing capacity of the parental population along with that of 36 plaque-derived subpopulations. Thirty-five of the plaque isolates were like the parental virus, i.e., they induced low yields of IFN that covered an 11-fold range, from 15 to 168 U/10^7 cells, with a standard deviation 2.4 times less than the mean IFN yield (Table 2). One of the plaque-derived populations was exceptional. Plaque isolate no. 22-20 induced 31,000 U of IFN per 10^7 cells. This phenotype has been stable over several passages at low multiplicity in GMK-Vero cells (31). (In an earlier publication VSV Lab code no. 22 was identified incorrectly as 387CRB [30]. The correct designation, 287CRB, indicates it was isolated in February 1987 in Costa Rica, and is of bovine origin.)

IFN-inducing capacity of VSV-NJ plaque-derived populations from a single lesion on an infected cow (1290CRB, no. 71). About 200 μl of fluid from a single vesicular lesion was obtained from the udder of a cow in Costa Rica naturally infected with VSV-NJ (case 4190, cow no. 63; 1290CRB, no. 71, Alajuela Province, Grecia, San Roque). This vesicular material was assayed directly at 1.4×10^9 PFP/ml on GMK-Vero cells. Seventy-seven plaques were picked, and about 10^4 PFP from each plaque isolate were used to infect a monolayer of 5×10^6 Vero cells to produce a working stock. The plaque isolates generated stocks with a range of titers from 0.4×10^9 to 3.2×10^9 PFP/ml with a mean (± standard deviation) of 1.2×10^9 (0.4×10^9). These working stocks were used to generate IFN induction dose-response curves in primary chicken embryo cells. Figure 4 is a histogram representing the plateau (maximum) yields of IFN induced by each of the 77 plaque-derived subpopulations from the parental virus. The IFN yields cover a ninefold range, from 310 to 2,800 U/10^7 cells, with a standard deviation 2 times less than the mean IFN yield (Table 2). The parental virus tested directly from the original vesicle induced a yield of 1,770 U/10^7 cells.

Figure 5 shows a normal plot for the frequency distribution of the logarithms of IFN yields for the plaque-derived subpopulations of VSV-NJ illustrated in Fig. 4. These data show a good fit to a Gaussian distribution when plotted as the logarithm of the IFN yield, where the mean ± standard deviation of the $\log_{10}$ IFN yield is 2.89 ± 0.18. The frequency distribution of IFN yields showed a poor fit to a Gaussian distribution when the arithmetic values for IFN yields were plotted directly (mean ± standard deviation, 858 ± 407). A similar plot of $\log_{10}$ IFN yields for plaque-derived subpopulations of VSV-NJ Lab code no. 11 (Fig. 2) also showed a good fit to a Gaussian distribution but a poor fit when arithmetic values were used (data not shown).

DISCUSSION

Isolates of VSV vary widely in their capacity to induce IFN, behavior which is consistent with quasispecies populations containing multiple genomic changes that produce a quantitatively variable phenotype. Stocks of VSV field isolates or laboratory strains generated in GMK-Vero cells as an IFN-neutral host, and tested in aged primary chicken embryo cells, revealed wild-type, non-temperature-sensitive parental populations that possessed IFN-inducing capacities that ranged from <10 to

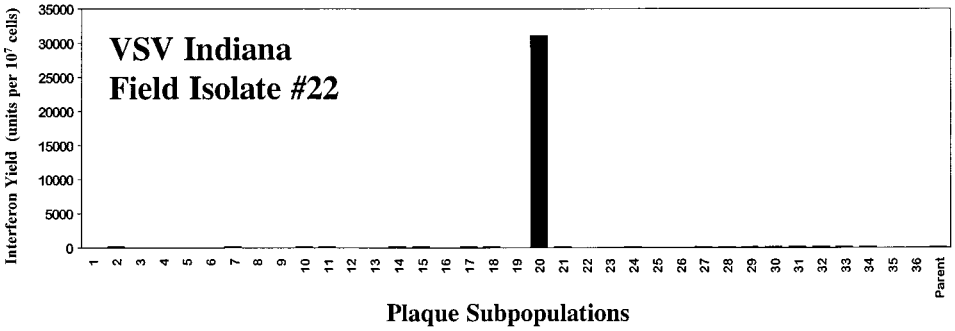

FIG. 3. Histogram which displays the maximum IFN yields generated in aged primary chicken embryo cell monolayers infected with 36 plaque-derived subpopulations of field isolate VSV-IN no. 22 (287CRB) in the order they were picked, along with the original parent. IFN yields of <70 U/ml are not resolved on this scale.

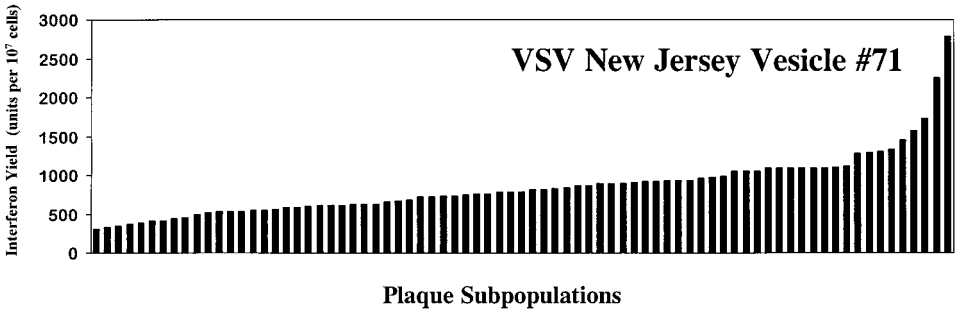

FIG. 4. Histogram which displays in an ascending order the maximum IFN yields generated in aged primary chicken embryo cells infected with 77 plaque-derived subpopulations from a single vesicle on the udder of a cow infected with VSV-NJ (no. 71, 1290CRB; cow no. 63, case 4190), along with that of the parent. Plaque isolate numbers not shown.

45,000 U/10^7 cells in this and other studies (42, 44). A comparison of 32 VSV-IN and 34 VSV-NJ field and laboratory isolates revealed an almost 10-fold difference in the average IFN-inducing capacity of the two serotypes. Many of these 66 isolates were separated widely in time (1925 to 1990), country of origin (United States to Panama), geographical location, and ecological area (tropical to temperate) (38). Uncloned field and laboratory isolates of the same serotype showed a standard deviation of the mean yield of IFN that was greater than the mean value, consistent with their diverse origins and unusual geographical stepwise evolutionary pattern (33).

Most VSV-IN isolates were characterized phenotypically as non- to low-yield inducers of IFN (Fig. 1a). They also were able to suppress IFN induction in coinfected cells otherwise programmed for induction (20, 26, 27). In contrast, all VSV-NJ isolates induced IFN and could be ordered in a continuum of IFN yields (Fig. 1b). As with the IFN-inducing isolates of VSV-IN, the NJ isolates generated type $r \geq 1$ IFN induction curves (20, 22). This implies that chicken cells infected with ≥ 1 IFN-inducing particles of VSV produce a full yield of IFN. Maintenance of the maximum yield of IFN when multiplicities of infection exceed 1 demonstrates that the population of VSV contains little, or no, suppressing activity other than that which regulates the yield of IFN characteristic of that isolate (14). Phylogenetic analyses have showed marked genetic stability of VSV-NJ genotypes within specific areas of endemicity, suggesting that ecological factors, such as insect vectors or reservoirs, rather than temporal factors influence virus evolution (38). In light of this observation, it is interesting to note that the four VSV-NJ field isolates (no. 62, 63, 59, and 4) that induced the least amount of IFN (550 $\pm$ 148 U/10^7 cells) are phylogenetically very closely related and originate from a premontane forest ecological zone in northern Panama, whereas the three VSV-NJ field isolates (no. 65, 52, and 51) that induced the most IFN (22,800 $\pm$ 19,300 U/10^7 cells), i.e., 40-fold more, are phylogenetically closely related to each other. They are more distantly related to the Panama viruses and originate from a tropical dry forest ecological zone in northwestern Costa Rica (4). If we assume that different vectors or reservoirs maintain each virus at each ecological zone, then the IFN induction phenotype might reflect the adaptation to such host(s) (15, 47).

In contrast to the behavior of different specimens of VSV-IN isolated over a wide range of time and location, where the standard deviation is greater than the mean of the IFN yields, the IFN yields induced by 27 plaque-derived subpopulations from a single IFN-inducing field isolate of VSV-IN (no. 11) (Fig. 2) show a standard deviation less than the mean (Table 2). Interestingly, the parent stock induced a lower than average yield of IFN. This is thought to reflect the downregulation of the yield of IFN from the better inducers in the population through the action in multiply infected cells of the IFN induction-suppressing activity intrinsic to the inducers of lower IFN yields (14). As calculated from the Poisson distribution, close to maximum yields of IFN are produced in a type $r \geq 1$ IFN induction dose-response curve when cells are infected with an average of four virions (22). At this multiplicity 91% of the cells are infected with ≥ 2 virions. Thus, cells coinfected with virions from a noncloned quasispecies population of VSV-NJ whose members intrinsically induce different yields of IFN may be downregulated to the level of the lowest level inducer in a given infected cell. This situation was observed when chicken cells were coinfected with both a high- and a low-level IFN inducer (14).

The relatively high standard deviation from the mean of the IFN yields that were induced by plaque isolates from a single stock (no. 11) reflected a range that differed 90-fold (Fig. 2). This broad range in IFN-inducing capacity is consistent with the high mutation rates responsible for quasispecies populations of VSV and the generation of genotypes with variable sequences. Interestingly, the subpopulations of this single isolate with its 90-fold range of induced IFN yields almost suffice to account for the broad range of IFN yields induced by the entire range of field isolates examined in this study from widely

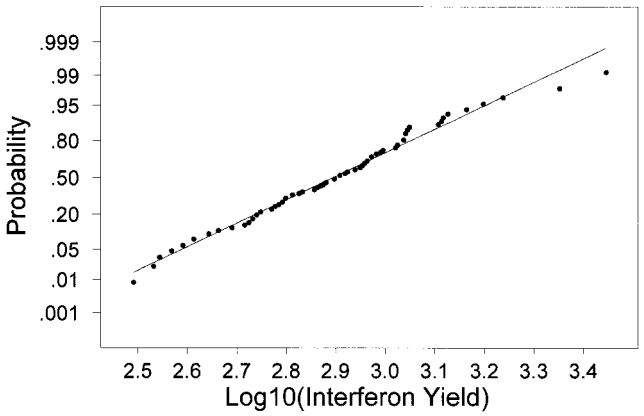

FIG. 5. A normal plot for the frequency distribution of the logarithm of IFN yields for the plaque-derived subpopulations of VSV-NJ illustrated in Fig. 4. Some of the data points represent duplicate or triplicate values and therefore are not represented as separate points on the figure; however, they have been averaged into the calculations for the mean and standard deviation. Mean, 2.89; standard deviation, 0.18; n, 77; R (Shapiro-Wilk test), 0.9922; P, >0.10 (approximately).

ranging times and geographical and ecological locations. Consequently, we propose that the IFN yield characteristic of a VSV field isolate most likely reflects its origin from a quasispecies population with an intrinsically wide range of IFN-inducing capacities and thus represents natural infection initiated with a single virus particle. This implies that the VSV populations that develop in infected animals result from genetic bottlenecks and consequently produce subpopulations which differ significantly from the consensus parental wild-type population (6). From this view, the appearance of IFN-inducing field isolates of VSV-IN, an unusual phenotype for this serotype, in outbreaks of vesicular stomatitis in Costa Rica and neighboring Panama in 1984 and 1985 (30), resulted from infection initiated by a rare IFN-inducing particle in the large repertoire of genomes in the quasispecies population of otherwise dominant non-IFN-inducing particles capable of suppressing IFN induction. This situation was anticipated by the general statement of Duarte and colleagues who noted that "whenever genetic bottlenecks of RNA viruses occur, enhanced biological differences among viral subpopulations may result" (11). Further support for this scenario comes from the observation that a stable subpopulation of an IFN-inducing particle of VSV-IN (no. 22-20) was plaque isolated from a wild-type field population that did not induce IFN when tested as a whole (Fig. 3). Once established as a plaque-derived subpopulation propagated in an IFN-neutral host cell, the IFN-inducing phenotype of VSV-IN no. 22-20 was stable, further supporting the view that multiple nucleotide changes may be required for the acquisition of IFN inducibility (43). The progeny of a non-IFN-inducing plaque-derived isolate (no. 22-25) from the same population has maintained its parental phenotype, comparable to the 34 other sibling subpopulations, over several passages. If the exceptional IFN-inducing plaque isolate no. 22-20 is excluded from calculations, the standard deviation of the mean for the IFN-inducing capacity of the remaining 35 isolates is less than the mean, as is the case for the other two plaque-derived subpopulations of VSV studied similarly (Fig. 2 and 4). Nonetheless, there is an 11-fold range in IFN yields induced by the plaque-derived subpopulations of VSV-IN field isolate no. 22, consistent with a variable phenotype accumulating in a quasispecies population. For this quantifiable phenotype, the landscape must be rich with a continuum of variants, with detection of the smallest incremental change being limited by our capacity to distinguish small differences in IFN yields.

The sampling of a single vesicle on the udder of a diseased cow represents the first time a virus population from a lesion of vesicular stomatitis has been plaque cloned directly to study a phenotype that can be measured quantitatively over a wide range of values. These 77 plaque-derived subpopulations can be ordered by their IFN-inducing capacities over a ninefold range of values (Fig. 4), and like other subpopulations related by plaque isolation from the same parent stock, they show a standard deviation that is less than the mean IFN yield (Table 2). The IFN-inducing capacity of the noncloned vesicle material was higher than the mean. As previously noted, the measured IFN-inducing capacity of a quasispecies population most likely reflects the complex interplay in the host cell of antagonistic IFN-inducing and IFN-suppressing activities possessed by an infectious particle of VSV (20) and not simply a weighted average of their individual IFN-inducing capacities.

The frequency distribution of IFN yields for the 77 plaque-derived subpopulations shown in Fig. 4 shows a good fit to a Gaussian distribution when plotted as the logarithm of the IFN yield, where the mean $\pm$ standard deviation of the $\log_{10}$ IFN yield are equal to 2.89 ± 0.18 (Fig. 5). The W-test for normality gave an R of 0.9922 and an approximate P value of >0.10. There was a slight deviation from a good fit for the two best IFN inducers. These same data showed a poor fit to a Gaussian distribution when the arithmetic values for IFN yields were plotted (data not shown), where the Shapiro-Wilk test for normality (45) gave an R of 0.9129 and an approximate P value of <0.01. When data for the IFN yields from plaque-derived subpopulations of VSV-IN no. 11 (Fig. 3) were plotted similarly, again there was a good fit to the ln IFN yield and a poor fit when arithmetic values were used (data not shown). These analyses suggest that the genomes of VSV populations may differ from the nominal parent (the virion that initiated plaque formation or infection in a host) in some incremental manner sufficient to elicit a comparable step-wise change in the IFN-inducing capacities of population members. The nature of the genomic changes responsible for the incremental differences observed in the IFN-inducing capacities of VSV subpopulations is as yet unknown. There are at least two variables at work, the antagonist activities of IFN induction and the suppression of IFN induction (20).

As documented, most VSV-IN isolates were characterized phenotypically as non- to low-yield inducers of IFN (Fig. 1a) but were able to suppress IFN induction in cells otherwise programed for induction (26). Indeed, a single virion of VSV-IN suffices to express this phenotype in chicken embryo cells, even following exposure of the virus to 50 lethal hits (2,625 ergs/mm^2) of UV radiation (254 nm) (27). This demonstrates that amplification of viral gene products is not necessary and that activities extant in the virion suffice for IFN induction suppression. Leader RNA and proteins N, P, and M of VSV-IN do not of themselves appear to be responsible for the virion's IFN induction-suppressing activity (31), although functional virion transcriptase is required (27). Transcripts of chicken mRNA fail to appear in cells infected with VSV-IN that do not induce IFN but do suppress its induction (41). Such viruses also fail to activate or induce in a timely manner the transcription factors NF-κB (4) and IRF-1 (17), which accumulate in chicken cells infected with VSV isolates that induce IFN. Nor does IRF-1 appear in chicken cells simultaneously infected with an IFN-inducing isolate and a non-IFN-inducing isolate expressing the dominant IFN induction-suppressing phenotype (17). This suggests that the inhibition of activation of specific transcription factors may underlie the IFN induction-suppressing phenotype and that inhibition of host cell transcription may contribute to the suppression of IFN induction (4, 13). From this view, the production of IFN characteristic of a VSV isolate represents the outcome of a race between the rate at which the IFN inducer moiety, dsRNA (21), forms and initiates induction of the IFN gene(s), including the activation of transcription factors (4, 5, 17), and the rate at which this latter activity is suppressed by a virion component(s) (31). The implication of multiple transcription factors in the regulation of IFN induction and suppression allows for a wide range of responses by the host cell as it responds to these two opposing actions with the resulting production of a characteristic yield of IFN. The host cell must also play a role in the expression of these phenotypes since the IFN-inducing capacity of VSV isolates as measured in chicken embryo cells is not expressed in some mammalian cells (29, 42).

The incremental changes that are documented here in the IFN-inducing capacities of VSV populations and subpopulations are consistent with the quasispecies nature of VSV populations (7–9, 16, 34, 36) and are thought to represent a direct measure of the genetic variability demonstrable in RNA virus populations as measured elegantly by others, for example, variations in fitness (5, 6, 10–12, 34–36) or in genomic sequences

(37). It is interesting to speculate that the observed continuum of IFN-inducing capacities represents cumulative changes in the genome that result in incremental effects on the two antagonistic attributes that define the observed phenotype. Studies to examine this possibility are directed at the transcriptase gene of VSV because of its large size and multifunctional capacity (1, 2) and the evidence that VSV plus strand leader RNA, and the products of genes N, P, and M, of themselves are not responsible for downregulating IFN gene activation in chicken embryo cells (31).

These studies have revealed a significant difference in the intrinsic IFN-inducing capacities of the Indiana and New Jersey serotypes of VSV. Further study is required to assess the role of this phenotype, if any, on the natural course of the disease. In this context, there appears to be no discernible differences between VSV-IN and VSV-NJ in the sequelae and course of vesicular stomatitis (37a). Since VSV-NJ accounts for about 90% of the clinical cases of vesicular stomatitis (39, 46), there may be some evolutionary advantage to the VSV-NJ serotype as it relates to cellular regulatory factors associated with the IFN induction phenotype and their expression in a vector or reservoir (38).

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