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INDUCTION OF INTERFERON α FROM HUMAN LYMPHOCYTES BY AUTOLOGOUS, DENGUE VIRUS–INFECTED MONOCYTES

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Monocytes appear to play an important role in dengue virus infection. They have been identified as cells that support dengue virus replication (1). Dengue virus has been recovered from circulating mononuclear cells and lymphoid organs of dengue virus–infected humans and monkeys (2–4). Cell separation experiments showed that dengue virus replicates in monocyte fractions, but did not replicate in the monocyte-depleted fractions (1). Furthermore, monocytes containing dengue virus–like structures have been detected in the skin and kidney of dengue hemorrhagic fever patients (5, 6). Therefore, dengue virus–infected monocytes may be active in inducing responses from immune-competent lymphocytes. It has been hypothesized that immune elimination of dengue virus–infected monocytes (DV-monocytes)[1] may lead to the severe complications of dengue infection, hemorrhagic manifestations, and shock, which are more commonly observed in secondary dengue infections (7).

In the report we analyzed the in vitro interaction between DV-monocytes and lymphocytes as a model of primary dengue infection. We used autologous monocytes and lymphocytes from dengue nonimmune donors. Lymphocytes produce high titers of interferon α (IFN-α) after being exposed to DV-monocytes. The IFN-α induced was able to inhibit infection of other monocytes by dengue virus. These results suggest that IFN induction from lymphocytes by DV-monocytes is an important defense mechanism in primary dengue infections.

Materials and Methods

Human Peripheral Blood Mononuclear Cells (PBMC)

Peripheral blood specimens were obtained from healthy blood bank donors from Massachusetts who did not have antibodies to dengue virus as determined in a plaque reduction neutralization test. PBMC were separated by the Ficoll-Hypaque density gradient centrifugation method. Cells were resuspended at the concentration of $1–3 \times 10^7$ cells/ml in RPMI 1640 (Flow Laboratories, Rockville, MD) containing 10% FCS and 10% dimethylsulfoxide (Fisher Scientific Company, Springfield, NJ) and were cryopreserved until use.

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[1] *Abbreviation used in this paper:* DV-monocyte; dengue virus–infected monocyte.

Virus and Anti-dengue 2 Antibody

Dengue virus type 2 New Guinea C strain was used for infection of monocytes. The seed virus was passed in mouse brain and then propagated in mosquito cells (C6/36). The virus pool titered 5×10^7 PFU/ml in Vero cells. Ascites fluid from mice hyperimmunized with dengue virus type 2 was used as anti-dengue 2 antibody. Virus and antibody were kindly supplied by Dr. Walter E. Brandt of Walter Reed Army Institute of Research, Bethesda, MD. The titer of this antibody was 1:1,024 in a plaque-neutralization test. Hyperimmune ascitic fluid was heated at 56°C for 30 min to destroy complement activity before use.

Titration of Dengue Virus

The amount of infectious dengue virus was determined by plaque titration in Vero cells. 0.1 ml of serially diluted culture supernatant fluid was placed on Vero cell monolayers in 24-well plates (Costar, Cambridge, MA) and incubated at 37°C for 2 h. The culture fluid was then removed, the cells were washed once with RPMI medium, and 1 ml of overlayer containing 1% agar (Difco Laboratories, Detroit, MI) in MEM, 10% FCS, 0.5% nonessential amino acids, 0.5% essential vitamins, and 0.3% DEAE-dextran was added. The plates were cultured at 37°C for 7 d, stained with neutral red, and the number of plaques was counted.

Isolation of Adherent Cells

2×10^7 PBMC in 4 ml of RPMI/10% FCS were cultured in a plastic petri dish with 6-cm diameter (Falcon Laboratories, Oxnard, CA) at 37°C for 20 h. The dish was rinsed several times with RPMI/10% FCS until no nonadherent cells were observed under an inverted microscope. These adherent cells were considered as monocytes. The purity of monocytes was >90% as determined by a phagocytic assay (8) and by membrane fluorescence staining using anti-Mo2 antibody (Coulter Immunology, Hialeah, FL).

Infection of Human Monocytes with Dengue Virus

Dengue virus (5×10^7 PFU/ml) was incubated with 1:10,000 diluted anti-dengue virus type 2 antisera at 4°C for 1 h. Monocytes were infected with dengue virus at a multiplicity of infection (m.o.i) of 5–10 PFU/cell at 37°C for 2 h on plastic petri dishes. Infected cells were washed once and cultured at the concentration of 10^6 cells/ml in RPMI/10% FCS for 48 h unless otherwise stated. The cells were collected and used as IFN-inducer cells.

Treatment of PBL with Actinomycin D

PBL (4×10^6 cells/ml) were incubated with various concentrations of actinomycin D (Sigma Chemical Co., St. Louis, MO) at 0.64 μg/ml at 37°C for 1 h to inhibit the production of IFN (9). Cells were washed three times and then were used as producer cells.

Treatment of DV-monocytes with Glutaraldehyde

Dengue virus–infected and uninfected monocytes were treated with glutaraldehyde as previously described (10). Cells were washed three times with PBS, treated with 0.025% glutaraldehyde in PBS for 10 min at room temperature, washed three times in PBS, resuspended in RPMI/10% FCS, and used as inducer cells. After treatment, all the cells were dead, as determined by trypan blue dye–exclusion test.

Treatment of DV-monocytes with Paraformaldehyde

Dengue virus–infected and uninfected monocytes were washed three times with PBS, treated with 3.5% paraformaldehyde in PBS for 25 min at room temperature, washed three times with PBS, resuspended in RPMI/10% FCS, and used as IFN inducer cells. After treatment, >97% of the cells were dead, as determined by trypan blue dye–exclusion test.

Induction of IFN

5×10^5 PBL were cultured with 10^4 autologous DV-monocytes in 0.2 ml of RPMI/10% FCS at 37°C for 20 h unless otherwise stated. After cultivation, 0.15 ml of the supernatants were harvested and examined for IFN activity.

mAbs and Other Reagents

Anti-HLA-DR antibody reacts with monocytes, B cells, and activated T cells (11). OKT3 antibody reacts with T cells (12). Anti-B1 and anti-Leu-12 antibodies react with B cells (13, 14). Anti-Leu-11 antibody react with NK cells and neutrophils (15). OKM1 antibody reacts with monocytes and NK cells (16). FITC-conjugated anti-HLA-DR, FITC-conjugated anti-Leu-12, and FITC-conjugated anti-Leu-11, were purchased from Becton Dickinson Co. (Mountain View, CA). Anti-OKT3 and anti-OKM1 were purchased from Ortho Diagnostic Co. (Raritan, NJ). FITC-conjugated anti-B1 was purchased from Coulter Immunology (Hialeah, FL). FITC-conjugated goat anti-human Ig (IgA + IgG + IgM) were purchased from Cappel Laboratories (West Chester, PA).

Cell Separation by FACS

Nonadherent PBL were stained by direct membrane immunofluorescence or by indirect membrane immunofluorescence. Stained lymphocytes were sorted with a FACS 440 (Becton-Dickinson Co.) as previously reported (17). Cell viability after sorting exceeded 95% by a trypan blue dye–exclusion test. The purity of each of the sorted populations was >96%.

Interferon Assays

Bioassay. Interferon was assessed by a cytopathic effect–reduction assay (18). Twofold serial dilutions of supernatant fluids obtained from PBL exposed to dengue virus–infected cells were incubated on human fibroblast cells (trisomic for chromosome 21) for 20 h at 37°C and then challenged with vesicular stomatitis virus. An international interferon standard was included in each assay, and the titers were read after 24 h at 37°C.

Radioimmunoassay. The IFN content of supernatant fluids were also measured in RIA specific for IFN-α and IFN-γ as previously described (10). Briefly, 6.5-mm-diam etched polystyrene beads coated with sheep polyclonal antiserum to IFN-α or -γ were incubated with IFN-containing samples for 2–3 h at 4°C, washed twice, and then incubated with either radioiodinated ^{125}I-MT4/E4 mAb to IFN-α2 (19) or ^{125}I-5J mAb to IFN-γ (20) for 16 h at 4°C. The beads were rewashed and counted in an LKB gamma counter. RIAs were calibrated in international units per milliliter using relevant international standards for HuIFN-α and HuIFN-γ. These mAbs used to detect IFN-α and IFN-γ are specific for IFN-α and IFN-γ, respectively.

Results

IFN Induction from PBL by Autologous DV-monocytes. To dissect the interaction between PBL and DV-monocytes, we cultured PBL and infected monocytes together or separately for 20 h, and examined the culture fluids for IFN activity. Culture fluids of PBL and those of DV-monocytes contained only low titers of IFN activity. When PBL and DV-monocytes were cultured together, IFN activities at titers of 200–1,200 U/ml were detected. Only very low titers of IFN were detected when PBL were cultured with uninfected monocytes (Table I). Time-course study showed that IFN activity was detected as early as 4 h after the beginning of incubation, and the IFN titers reached a maximum level at 16 h. To confirm that the IFN detected was produced by PBL, the PBL were treated with a 0.64 μg/ml of actinomycin D and then cultured with DV-monocytes. Pretreatment of PBL with actinomycin D decreased the titer of IFN

TABLE I

Induction of IFN from PBL by Autologous DV-monocytes

Culture	Donor:	IFN (U/ml)					
		A	B	C	D	E	F
PBL + DV-monocytes*		800	400	200	300	1,200	400
PBL + uninfected monocytes		38	50	6	9	<6	<6
PBL		18	50	6	<6	9	<6
DV-monocytes		18	6	18	<6	75	12
Uninfected monocytes		<6	<6	<6	<6	<6	<6

5×10^5 PBL were cultured with 10^4 autologous DV-monocytes for 20 h. Culture fluids were examined for IFN activity by bioassay.

* The percentages of dengue antigen–positive cells were as follows: donor A, 63%; donor B, 57%; donor C, 40%; donor D, 45%; donor E, 58%; donor F, 49%.

TABLE II

Abrogation of IFN Production by Treatment of PBL with Actinomycin D

PBL	Inducer Cells	IFN (U/ml)
Untreated	DV-monocytes*	800
Actinomycin D–treated	DV-monocytes	50
Untreated	Uninfected monocytes	6
Actinomycin D–treated	Uninfected monocytes	12
Untreated	None	12
Actinomycin D–treated	None	6

5×10^5 PBL were cultured with 10^4 inducer cells for 20 h. PBL were treated with actinomycin D at 0.64 μg/ml as stated in Materials and Methods.

* Percentage of dengue antigen–positive cells was 58%.

detected from 800 U/ml to 50 U/ml without changing the viability of PBL (Table II). This result confirmed that the IFN detected in the cultures containing both PBL and DV-monocytes was produced by PBL.

Requirement for Cell Contact Between PBL and DV-monocytes for IFN Induction. Culture fluids from DV-monocytes were examined for the ability to induce IFN from PBL. When PBL were incubated in the culture fluids of DV-monocytes for 20 h, no IFN activity was induced from PBL. These DV-monocytes used in the experiment, however, induced 200 U/ml of IFN from PBL when they were cultured with PBL.

Experiments were also performed using a microporous membrane with 0.4-μm pores (Costar, Cambridge, MA) to separate the PBL and DV-monocytes. When PBL and dengue virus–infected cells were mixed and cultured, high titers of IFN were detected. However, when PBL and DV-monocytes were cultured in the same well but separated by the membrane, only a low titer of IFN was detected (Table III). These results indicate that cell contact between PBL and DV-monocytes was required for IFN induction.

No Requirement for MHC Compatibility. To determine whether IFN induction

TABLE III
Requirement for Cell Contact Between PBL and DV-monocytes for Induction of IFN

Culture	Cell contact between PBL and monocytes	IFN (U/ml)
PBL + DV-monocytes*	Yes	800
PBL + DV-monocytes	No	50
PBL + uninfected monocytes	Yes	12
PBL + uninfected monocytes	No	25
PBL alone	—	12
DV-monocytes	—	12
Uninfected monocytes	—	<12

4×10^6 PBL and 10^5 DV-monocytes were cultured in 1 ml of RPMI/10% FCS in the same well together or separated by a microporous membrane for 20 h. Culture fluids were examined for IFN activity.
* Percentage of dengue antigen–positive cells was 29%.

TABLE IV
No Requirement for MHC Compatibility for IFN Induction

PBL	Inducer monocytes	IFN (U/ml)
Donor 1*	Donor 1	
	Infected‡	200
	Uninfected	<3
	Donor 2	
	Infected‡	200
	Uninfected	<3
Donor 2*	Donor 1	
	Infected	200
	Uninfected	<3
	Donor 2	
	Infected	300
	Uninfected	<3

5×10^5 PBL from donors 1 and 2 were cultured with 10^4 inducer cells from donors 1 and 2.
* HLA typing of donors; donor 1: A1, A9, B5, B8, CW6, DR3, DR5; donor 2: A2, A28, B35, BW55, CW3, CW4, DR4.
‡ Percentages of dengue antigen–positive cells in infected monocyte were as follows: donor 1, 43%; donor 2, 53%.

is restricted by MHC, we analyzed IFN induction using the PBL of two donors who have completely different MHC class I and class II antigens. The DV-monocytes of each donor induced similar titers of IFN from the PBL of either donor (Table IV). These results indicate that MHC compatibility is not required for IFN induction from PBL by DV-monocytes.

IFN Induction by DV-monocytes Treated with Paraformaldehyde or Glutaraldehyde. We then examined the ability of paraformaldehyde-treated DV-monocytes to induce IFN. These cells retained the ability to induce IFN from PBL (Table V). Glutaraldehyde-treated, DV-monocytes also induced IFN from PBL (data not presented). DV-monocytes treated with paraformaldehyde or glutaraldehyde

TABLE V

Induction of IFN by Paraformaldehyde-fixed DV-monocytes

Culture	IFN (U/ml)	
	Untreated inducer cells	Paraformaldehyde-fixed inducer cells‡
PBL + DV-monocytes*	300	150
PBL + uninfected monocytes	<6	<6
PBL	<6	<6
DV-monocytes	25	<6
Uninfected monocytes	<6	<6

5×10^5 PBL were cultured for 20 h with 10^4 inducer cells untreated or fixed with paraformaldehyde.

* Percentage of dengue antigen–positive cells was 34%.

‡ Treatment of monocytes with paraformaldehyde is described in Materials and Methods.

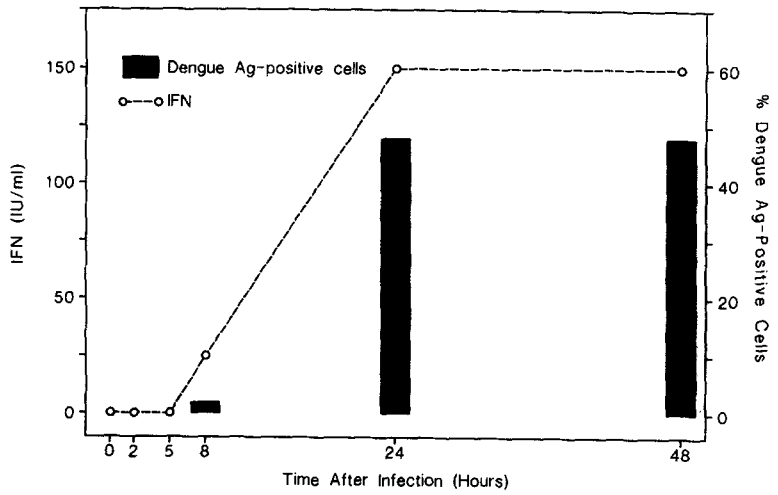

FIGURE 1. Correlation between the appearance of dengue antigen (*Ag*) in DV-monocytes and IFN-inducing ability. DV-monocytes were fixed with paraformaldehyde at various times after infection. 5×10^5 PBL were cultured with 10^5 glutaraldehyde-fixed DV-monocytes for 20 h.

did not produce IFN or infectious dengue virus. These results indicate that production of IFN is due to stimulation of PBL by dengue virus–infected cells and does not directly require infectious dengue virus.

Correlation Between Appearance of Dengue Antigen in Infected Monocytes and IFN-inducing Ability. We then analyzed DV-monocytes at various times after infection to determine whether there is a correlation between the ability of infected cells to induce IFN and dengue antigen expression. At various times after infection, dengue-infected cells were fixed with paraformaldehyde and used as inducer cells. The infected monocytes were able to induce IFN 8 h after infection, when dengue antigens became detectable. The IFN-inducing ability reached a maximum level 24 h after infection, when the percentage of antigen-positive cells also reached a maximum level (Fig. 1). These results indicate that there is a

strong correlation between antigen expression and IFN-inducing activity of dengue-infected cells.

Characterization of IFN-producing Cells Using mAbs. PBL that produce IFN in response to autologous dengue virus–infected monocytes were characterized using six mAbs; anti-HLA-DR, OKT3, anti-Leu-12, anti-B1, anti-Leu-12, and OKM1 antibodies, and one polyclonal anti–human immunoglobulin (IgA + IgG + IgM) antibody. PBL were positively enriched by FACS after reaction with these antibodies, and then were cultured with DV-monocytes. HLA-DR$^+$ and T3$^-$ cells produced high titers of IFN, while HLA-DR$^-$ and T3$^+$ cells produced very low or undetectable levels of IFN. B cells, characterized as sIg$^+$, B1$^+$, and Leu-12$^+$ produced some IFN, while sIg$^-$, B1$^-$, and Leu-12$^-$ cells also produced IFN. NK cells, characterized as Leu-11$^+$, produced a similar titer of IFN as Leu-11$^-$ cells. M1$^+$ cells, which also include NK cells, produced a higher titer of IFN than M1$^-$ cells (Table VI). These results indicate that IFN producer cells are heterogeneous, but the predominant IFN-producer cells are HLA-DR$^+$ and T3$^-$ cells, and that B and NK cell fractions also include some IFN-producing cells.

Characterization of Produced IFN. The IFNs produced were characterized by RIA using mAbs to IFN-α and IFN-γ as detector reagents. The predominant IFN induced from PBL by DV-monocytes was IFN-α; in addition, low titers of IFN-γ were also detected in some experiments (Table VII).

Inhibition of Dengue Virus Infection of Monocytes by Induced IFN. The effect of the induced IFN on dengue virus infection of monocytes was examined. PBL were cultured with DV-monocytes or with uninfected monocytes for 20 h, and culture fluids were collected and used in the following experiments. Syngeneic monocytes were incubated in the collected culture fluids for 20 h and then infected with dengue virus. None (out of 200) of the monocytes treated with culture fluids from PBL exposed to DV-monocytes, which contained IFN activity at 800 U/ml, contained dengue virus antigens; however, 67% (136 of 204) of the monocytes treated with culture fluids of PBL that had been exposed to uninfected monocytes contained dengue antigens, and a similar percentage (142 of 212) of monocytes with dengue antigens was observed without addition of supernatant fluids. A reconstitution experiment was performed in which 400 U/ml of Sendai virus–induced IFN-α (Interferon Sciences, Inc., New Brunswick, NJ) was added to monocyte culture 20 h before dengue virus infection. The percentage of dengue virus–infected cells decreased from 48 to 0.8%. These results indicate that IFN-α produced by PBL after exposure to DV-monocytes inhibits infection of monocytes by dengue virus.

Discussion

In this paper we report that DV-monocytes induce high levels of IFN production from autologous lymphocytes of nonimmune donors. It has been reported that monocytes actively replicate dengue virus (1); therefore, in vitro interactions between DV-monocytes and autologous PBL may reflect interactions that occur in vivo during dengue virus infection. Narayan et al. have reported IFN induction from PBL by lentivirus-infected monocytes of goats and sheep (21). Cell mixtures of PBL and monocytes were required for induction of IFN. MHC-compatibility of PBL and monocytes were not required in the lentivirus system (21). Those

TABLE VI
Characterization of PBL that Produce IFN in Response to DV-monocytes

Donor	IFN (U/ml)			
	Lymphocytic fractions	DV-monocytes	Uninfected monocytes	No inducer cells
A	DR^+	388	<6	<6
	DR^-	38	<6	<6
B	DR^+	382	<6	<6
	DR^-	7	<6	<6
A	$T3^+$	5	<3	<3
	$T3^-$	200	<3	<3
B	$T3^+$	<6	<6	<6
	$T3^-$	275	<6	<6
B	sIg^+	14	<3	<3
	sIg^-	200	<3	<3
C	$B1^+$	25	<6	<6
	$B1^-$	175	<6	<6
A	Leu-12^+	124	<9	<9
	Leu-12^-	75	<9	<9
B	Leu-12^+	150	<6	<6
	Leu-12^-	100	<6	<6
A	Leu-11^+	100	<6	<6
	Leu-11^-	100	<6	<6
B	Leu-11^+	100	6	<6
	Leu-11^-	150	<6	<6
A	$M1^+$	262	<6	<6
	$M1^-$	37	<6	<6
B	$M1^+$	200	<6	<6
	$M1^-$	75	<6	<6

PBL were sorted by FACS after reaction with mAbs. 3×10^5 sorted PBL were cultured with 10^4 inducer cells. Purity of FACS-sorted cells was >96%. The titers of IFN produced in the DV-monocyte cultures without PBL were subtracted from the titers produced in culture that contained PBL exposed to infected monocytes.

TABLE VII
Characterization by RIA of IFN Induced from PBL by DV-monocytes

Culture	IFN (U/ml) determined by RIA							
	Donor A		Donor B		Donor G		Donor H	
	IFN-α	IFN-γ	IFN-α	IFN-γ	IFN-α	IFN-γ	IFN-α	IFN-γ
PBL + DV-monocytes	300	14	1,000	2	150	<2	250	4
PBL + uninfected monocytes	<3	4	<3	5	<3	ND	<3	<1
PBL	<3	12	<3	<1	9	ND	<3	<3
DV-monocytes	<3	<1	17	<1	8	ND	<3	<3
Uninfected monocytes	<3	<1	12	<1	<3	ND	<3	<3

5×10^5 PBL were cultured with 10^4 DV-monocytes for 20 h. IFN contained in the culture fluids was determined by RIA.

results are very consistent with our results using human PBL, monocytes, and dengue virus, which belongs to the flavivirus group. Narayan et al. attributed the IFN induction to an IFN-inducing factor that was generated in the lentivirus-infected monocytes. We have not precisely identified what is responsible for inducing IFN by the DV-monocytes. However, de novo synthesized viral components in the infected cells appear to be necessary, because DV-monocytes acquired the ability to induce IFN ~8 h after infection, when dengue viral antigens were first detected in the infected cells. Infectious dengue virus produced by the infected monocytes was not responsible for inducing IFN, because DV-monocytes treated with paraformaldehyde or glutaraldehyde, which produced no infectious virus, also induced IFN from PBL. The presence of IFN-inducing factors secreted from dengue-infected monocytes is not likely, because IFN induction required cell contact between PBL and DV-monocytes, and the culture fluids of DV-monocytes did not induce IFN from PBL. Although the mechanism of IFN induction by lentivirus-infected goat monocytes may be similar to that by DV-monocytes, the IFN-producer cells and the IFN produced appear to be different. Narayan et al. characterized the IFN producer cells as T cells, and the IFN produced was different from the IFN produced by parainfluenza, which was probably IFN-α, or that induced by Con A, which was probably IFN-γ (21). In the present experiment, using human DV-monocytes and PBL, the IFN-producing cells were non-T cells, and the predominant IFN produced is IFN-α.

Characterization of the PBL that produce IFN after virus infections is a subject of controversy. The IFN-producing cells have been characterized as NK cells and large granular lymphocytes using density gradient cell separation techniques (22). Recently IFN-producing cells have been characterized using mAbs. Perussia et al. reported that the predominant IFN-producing cells after infection with influenza virus, Newcastle Disease virus, and Sendai virus were HLA-DR$^+$ and had no other surface markers (23). They also stated that NK cells and B cells produced lower levels of IFN. In those experiments they added virus to cultured PBL. In our experiments, we have used DV-monocytes as inducer cells. The phenotypes of the IFN-producing cells that Perussia et al. reported are very similar to the phenotypes of the IFN-producing cells in our experiments.

Identification of viral antigens responsible for IFN induction remains to be elucidated. Ito et al. reported that isolated hemagglutinin-neuraminidase glycoproteins of Sendai virus induce IFN from immune lymphocytes (24). Lebon reported that mAb to glycoprotein D of herpes simplex virus (HSV) inhibited the HSV-induced IFN production by PBL (25). We will perform similar experiments with purified dengue proteins and mAbs to identify the components of DV-monocytes that induce PBL to produce high levels of IFN.

The role of IFN in dengue virus infections remains to be elucidated. We have shown that culture fields obtained from PBL exposed to DV-monocytes completely inhibited dengue virus infection of monocytes. This result strongly suggests that the IFN-α produced by PBL in response to DV-monocytes plays a role in controlling primary dengue infection. In addition to antiviral effects, IFNs have immunoregulatory effects on NK cells (26), T cells (27), and B cells (28). Monocytes may also regulate immune responses to dengue virus by pre-

senting dengue antigens to T cells and by producing IL-1. Thus, the roles of IFN and monocytes in recovery from dengue virus infections and in the pathogenesis of dengue hemorrhagic fever and dengue shock syndrome are important subjects for further investigation.

Summary

Human monocytes actively replicate dengue virus. To dissect the primary immune responses to dengue virus-infected monocytes (DV-monocytes), we analyzed the interaction between autologous DV-monocytes and the peripheral blood lymphocytes (PBL) of dengue nonimmune donors. Interferon (IFN) activity was detected when PBL were cultured with DV-monocytes. Cell contact between PBL and DV-monocytes was required for IFN production; however, MHC compatibility between PBL and monocytes was not necessary. DV-monocytes fixed with paraformaldehyde or glutaraldehyde, which produced no infectious virus, also induced high levels of IFN from PBL. The ability of DV-monocytes to induce IFN correlated with the appearance of dengue antigens.

The PBL that produce IFN were characterized by FACS sorting using monoclonal and polyclonal antibodies. HLA-DR$^+$ and T3$^-$ cells produced high titers of IFN, while HLA-DR$^-$ and T3$^+$ cells produced very low or undetectable levels of IFN. Moderate titers of IFN were produced by cells contained in B cell fractions (surface immunoglobulin–positive, B1$^+$, and Leu-12$^+$), and cells contained in natural killer cell fractions (Leu-11$^+$ and OKM1$^+$). Therefore, IFN-producing cells are heterogeneous, and the predominant producer cells are characterized as HLA-DR$^+$ and non-T lymphocytes. The IFN produced was characterized by RIA using mAbs to IFN-α and IFN-γ. The IFN-α was the predominant IFN produced; in addition, a low level of IFN-γ was also detected in some experiments. The culture fluids obtained from PBL exposed to autologous DV-monocytes, which contained high IFN activity, completely inhibited dengue virus infection of monocytes. These results suggest that IFN-α produced by PBL exposed to DV-monocytes may play an important role in controlling primary dengue virus infection.

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