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Transmission of dengue virus-induced helper signal to B cell via macrophages

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Summary. The helper T cells (TH) generated in dengue type 2 virus (DV) infection of mice produce a soluble helper cytokine (HF) which enhances the clonal expansion of DV-specific IgM antibody plaque forming cells (PFC). The present study was undertaken to investigate the mechanism of transmission of the helper signal from TH and HF to B cells. It was observed that TH could transmit the helper signal to B cells by direct cell to cell contact, but HF could not do so without the presence of live macrophages (Mϕ). HF was adsorbed by both heat killed and live Mϕ but the former could not transmit it to B cells. Both the polypeptide chains of HF bind to Mϕ. HF remains on the surface of Mϕ and can be retrieved completely by contact with B cells for 40 min. The helper signal from TH or HF-adsorbed Mϕ could not be transmitted to B cells when they were separated from each other by a cell impermeable membrane. The enhancement of PFC count is greater when the signal is transmitted by HF-adsorbed Mϕ as compared to that by TH alone. Thus, even with lower frequency of TH a significant number of B cells may be triggered with the help of HF and Mϕ. The findings thus show that the DV-specific helper signal could be transmitted only by a close physical contact of the plasma membranes of the signal presenting cells (TH or HF-adsorbed Mϕ) and B cells.

Keywords: helper T cells, helper cytokine, helper signal transmission, macrophages

Dengue type 2 virus (DV) infection in mice induces generation of T helper cells (TH) which produce a soluble helper cytokine (HF) which enhances the clonal expansion of DV-specific IgM antibody forming cells. HF is a heat, acid and alkaline pH-labile protein molecule and acts in an antigen-specific and H2 restricted manner. Chromatographically purified HF has a molecular weight of 65–68 kDa on SDS-polyacrylamide gel electrophoresis. HF-specific antisera raised in mice inhibit its activity and react specifically with it in a Western blot assay and bind with it in an immunosorbent column. HF is composed of two disulphide bonded polypeptide chains, one chain having antigen and the other having I-A determinants; presence of both the chains is essential for the helper activity (Chaturvedi *et al.* 1985; 1987; 1991; 1992; Pahwa *et al.* 1988). Murine helper T cell clones, TH1 and TH2, secrete different cytokines some of which are excellent helpers of B cell response. TH1 clones secrete interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumour

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necrosis factor-β (TNF-β), while TH2 secrete IL-4, IL-5 and IL-6 (reviewed by Mosmann & Coffman 1989). HF differs from these cytokines in having higher molecular weight, dimeric structure (IL-5 occurs as dimer) having two polypeptide chains, antigen-specificity and H2-restricted action. Recently, Guy *et al*, (1989) have demonstrated a 500 kDa cell-free product of TH cells, a cell-free form of TCR $\alpha\beta$ dimer, which activates B cell antibody response. HF is similar to this product in having dimeric form, two polypeptide chains, antigen-specificity and H2-restriction, but differs from it in having a smaller molecular weight.

These findings raised the question of transmission of helper signal from TH to B cells via soluble HF. Macrophages (Mϕ) play varied roles in DV-infection; on the one hand they are the principal cell to replicate DV, and on the other hand they are obligatory for the processing and presentation of the DV antigen to B cells leading to clonal expansion of specific antibody forming cells (Chaturvedi *et al*, 1983; Rizvi *et al*, 1987, 1989). Further, the presence of live Mϕ is obligatory for the transmission of DV-induced suppressor signal from TS_1-SF to TS_2 suppressor cells; SF-adsorbed Mϕ transmit the signal to T-cells to recruit TS_2 by close physical contact of the plasma membranes of the two interacting cells (Chaturvedi *et al*, 1982; Shukla & Chaturvedi 1982; 1983). The present study, undertaken to resolve this question, indicates that DV-induced helper signal could be transmitted by TH or by HF-adsorbed Mϕ to B cells via close contact of the plasma membrane of the two interacting cells.

Materials and methods

Animals

Inbred adult Swiss albino mice, aged 3–4 months, obtained from the colony maintained in this Department, were used.

Virus

Dengue type 2 virus (DV), strain P23085, was kindly supplied by the Director, National Institute of Virology, Pune and was used as described elsewhere (Chaturvedi *et al*, 1977).

Chemicals and reagents

The special chemicals and reagents used were dithiothreitol (DTT), iodoacetamide and foetal bovine serum (Sigma Chemical Co., St Louis, USA); ultra-pure methanol (Spectrochem Pvt. Ltd, Bombay); Eagle's minimum essential medium (MEM, Microlabs, Bombay).

Preparation of helper T cells (TH) and the helper cytokine (HF)

DV-specific TH were generated as described (Chaturvedi *et al*, 1985, 1987). Briefly, groups of mice were injected with 100 LD_{50} of DV i.v. followed by harvesting of the spleens on the 4th day. A single cell suspension of spleens was prepared in MEM containing 5% foetal bovine serum. T cell enriched population was obtained by nylon wool column filteration (Julius *et al*, 1973) and used as TH. The HF was prepared and purified from the TH as described (Chaturvedi *et al*, 1991, 1992). The single cell suspension of the above spleens was prepared in phosphate buffered saline, pH 7.2 (PBS) and cultured in 5-cm glass Petri dishes at 37°C in presence of 5% CO_2. After 24 h the cultures were centrifuged at 3000 *g* for 10 min and the clear supernatant was collected and stored at −70°C. It was purified by low pressure liquid chromatography using Sephacryl S-200 column. The protein peak having helper activity was freeze dried in a Speed Vac (Savant Instruments, Inc., New York) and stored at −70°C in small aliquots. The protein content of the preparations was estimated by the method of Lowry *et al.* (1951).

Separation of chains of HF

Details of the separation of the two chains of HF has been described elsewhere (Chaturvedi *et al*, 1991). In brief, HF was treated with 5 mM DTT for 45 min followed by treatment with 20 mM iodoacetamide for 20 min. All the reactions were carried out at room temperature and in presence of 0.15 M Tris-HCl buffer. The preparation was run on Pharmacia high pressure liquid chromatography (HPLC) using reversed phase C18 column and isocratic methanol gradient. The preparation yielded two peaks which were collected separately and freeze dried.

Preparation of macrophages (Mϕ) cell sheet

Mouse peritoneal lavage cells were collected in 5 ml heparinized Eagle's minimum essential medium (MEM) containing 5% foetal bovine serum and layered in 5-cm glass Petri dishes. After incubation at 37°C for 2 h in the presence of 5% CO_2, the glass-non-adherent cells were washed off with Hank's basal salt solution (HBSS). The glass-adherent cell sheet thus obtained was considered Mϕ as described (Chaturvedi *et al*, 1982).

Adsorption of HF on Mϕ

The Mϕ cell sheet was layered with 20 μg HF dissolved in 1 ml PBS and incubated for 1 h at 37°C. Unadsorbed HF was removed by washing the cell sheet three times with HBSS. The cells were scraped off if required or the HF-adsorbed cell sheet was used as such.

Preparation of B-cell enriched subpopulation

Spleens were teased out with forceps in chilled MEM containing 5% foetal bovine serum and a single cell suspension was prepared. Mϕ were depleted from the cell suspension by treatment with carbonyl iron (Lymphocyte Separator reagent, Technicon Instruments Corpn, NY) and magnet. The cell suspension was filtered through nylon wool columns to obtain a B lymphocyte enriched subpopulation (Julius *et al*, 1973; Trizio & Cudkowicz 1974).

Assay of helper activity

The helper activity was assayed by counting DV-specific IgM antibody plaque forming cells (PFC) in the spleens of mice by the technique of Jerne and Nordin (1963) as described (Chaturvedi *et al*, 1985, 1987). Mice were inoculated with cyclophosphamide (200 mg/kg body weight) i.p. to remove precursors of endogenous T helper cells (Shand & Liew 1980), followed 24 h later with 1000 LD_{50} of DV i.p. and 48 h later still with HF i.v. Mice were killed 72 h after DV inoculation, the spleens were removed aseptically and a single cell suspension was prepared. Multiple slides were prepared from each mouse spleen and DV-specific PFC were counted.

Data obtained from the repeated experiments using 8–10 mice have been presented as mean ± s.e.m. after deducting background values. The data have been analysed using Student's *t* test for *P* value. A *P* value of less than 0.05 was considered significant.

Results

Direct effect of HF on B cells

This experiment was done to find out if HF acts directly on B cells or via Mϕ. An enriched subpopulation of B cells was obtained from DV-primed mice spleen cells and divided into various sets. To one set of B cells (8×10^6) 20 μg of HF was added; in the second set, HF adsorbed normal mouse Mϕ (8×10^6 cells) were layered over with B cells; to the third set only normal Mϕ were added; and the fourth set consisted only of B cells for control. In addition, the other sets consisted of DV-primed total spleen cells as such or inoculated with 20 μg of HF. All the sets were cultured for 24 h at 37°C in presence of 5% CO_2 and then DV-specific IgM PFC were counted. The data presented in Fig. 1 show that the PFC count in the B cells was

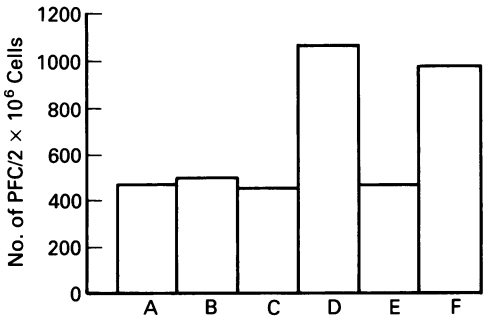

Fig. 1. Effect of HF on B cell enriched population obtained from DV-primed mice. A, B cells as such for control (0% help); B, B cells inoculated with HF (6%); C, B cells layered over normal Mϕ (−4%); D, B cells layered over HF-adsorbed Mϕ (127%); E, total spleen cells from DV-primed mice as control (0%); F, total spleen cells from DV-primed mice inoculated with HF (109%). All the sets were incubated for 24 h at 37°C in presence of 5% CO_2 and then DV-specific PFC counted. The percentage increase in help was calculated as follows:

Increase in help (%) =
100 × (PFC count of HF treated cells − background value)/{PFC count in untreated cells (control) − background value} − 100

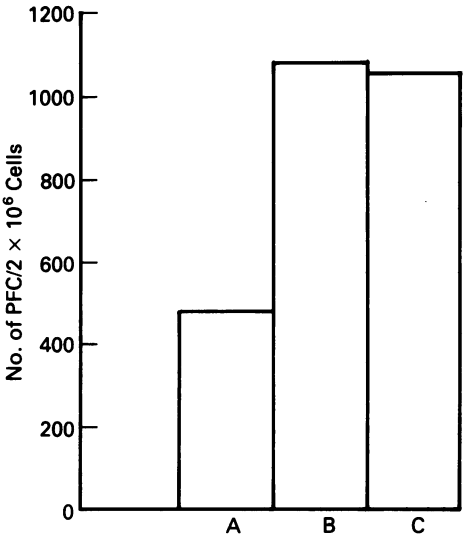

Fig. 2. Adsorption of HF on macrophages. DV-primed spleen cells were depleted of Mϕ and A, layered over normal Mϕ cell sheet as control (0% help); B, on HF-adsorbed Mϕ cell sheet (123%); and C, on normal Mϕ cell sheet followed by inoculation of HF (118%). All the sets were incubated for 24 h at 37°C and PFC counted. The increase in help was calculated as in Fig. 1.

$469 \pm 35/2 \times 10^6$ cells, while that in B cells treated with HF was $498 \pm 76/2 \times 10^6$. When the B cells were treated with HF in presence of normal Mϕ the counts were $1064 \pm 70/2 \times 10^6$, the increase in help being 127%. The counts in total DV-primed spleen cells were $466 \pm 34/2 \times 10^6$ which increased to $974 \pm 98/2 \times 10^6$ by inoculation of HF. This shows that macrophages are necessary for the action of HF.

Adsorption of HF on macrophages

This experiment was carried out to find whether HF is adsorbed on Mϕ. HF (20 μg) was adsorbed on normal mouse Mϕ cell sheet for 1 h at 37°C. The cell sheet was washed three times and layered with DV-primed spleen cells depleted of Mϕ. In the second group HF was inoculated to the Mϕ-depleted spleen cells layered over normal Mϕ. The cells were cultured for 24 h at 37°C in presence of 5% CO_2 and the PFC were counted.

The results presented in Fig. 2 show that HF adsorbed on Mϕ provided 123% help and that added with spleen cells provided 118% help. This shows that HF is adsorbed on Mϕ.

Live Mϕ mediated the action of HF

Mϕ cell sheet were killed by incubation at 60°C for 30 min. HF was adsorbed on these Mϕ. In one group Mϕ-depleted DV-primed spleen cells were cultured for 24 h with such Mϕ. In the second group, total DV-primed spleen cells were cultured. The cultures were harvested and PFC were counted after 24h. The results show that in the second group, where total spleen cells were added the increase in help was 128% while for the first set, which had Mϕ depleted spleen cells, the

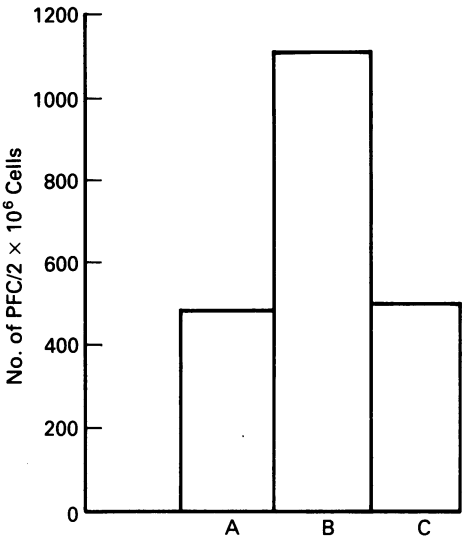

Fig. 3. The helper signal is transmitted only by live Mϕ and not by killed Mϕ. DV-primed spleen cells were depleted of Mϕ and layered over A, heat killed Mϕ cell sheet as control (0% help); C, heat killed HF-adsorbed Mϕ cell sheet (2%); B, Heat killed- HF- adsorbed Mϕ cell sheet was layered over by DV-primed total spleen cells (128%). All the sets were incubated at 37°C for 24 h and PFC counted. The increase in help was calculated as in Fig. 1.

help was 2% (Fig. 3). This suggests that Mϕ take active part in mediating the action of HF.

Both the chains of HF are adsorbed on Mϕ

HF has a disulphide bonded double chain structure, one chain having antigen and the other having H2-A determinants. For mediation of the activity, presence of both the chains of HF is obligatory. The present experiment was done to investigate the binding of the two chains individually to the Mϕ (Chaturvedi *et al.* 1991). In the first set the Mϕ monolayer was incubated with the α-chain of HF for 1 h at 37°C. The Mϕ monolayer was washed to remove the unadsorbed α-chain and then incubated with the β-chain at 37°C for 1 h. After washing, these Mϕ were scraped off and inoculated i.v. in

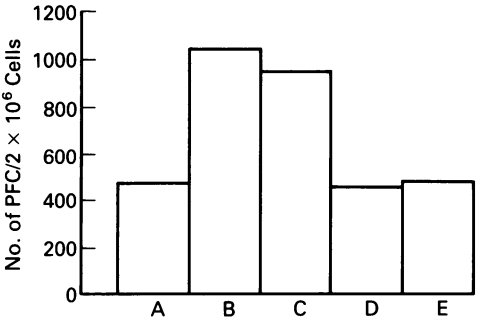

Fig. 4. Adsorption of the two polypeptide chains on Mϕ. A, DV-primed spleen cells as control (0% help); B, α-chain of HF was adsorbed on Mϕ followed by adsorption of the β-chain (119%); C, β-chain was adsorbed on Mϕ followed by α-chain (101%); D, α-chain adsorbed Mϕ (−4%); E, β-chain adsorbed Mϕ (2%). The Mϕ sheet of sets B to E was layered over by DV-primed spleen cells. All the sets were cultured for 24 h at 37°C and PFC counted. The increase in help was calculated as in Fig. 1.

DV-primed mice to assay the helper activity. After 24 h of injection the PFC were counted. In the second set β-chain was added first followed by α-chain. For control in one group only α-chain was added and in other only β-chain was added. The findings summarized in Fig. 4 show that when α-chain was added first followed by β-chain the increase in help was 119%. Similarly, when adsorption of β-chain on Mϕ was followed by α-chain the increase was 101%. When either α or β-chain alone was adsorbed there was no increase of helper activity, being −4 and 2% respectively. This showed that both chains of HF have the capacity to bind to Mϕ.

Retrieval of helper signal from HF adsorbed macrophages

In this experiment an effort was made to investigate whether the HF after binding to Mϕ remains at the surface or is internalized. This was done by a sequential retrieval of the helper signal by repeated exposure to DV-primed spleen cells. For this the HF-adsorbed Mϕ cell sheet was layered with DV-primed

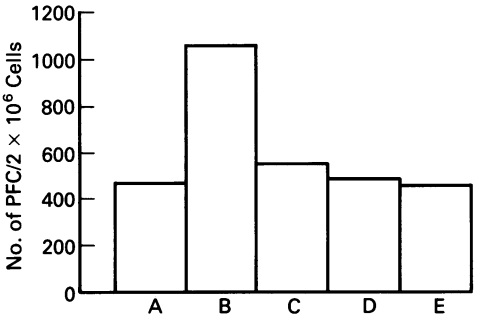

Fig. 5. Retrieval of the helper signal from HF-adsorbed Mϕ. DV-primed spleen cells were A, cultured as such for control (0% help); or layered over on HF-adsorbed Mϕ cell sheet and incubated at 37°C in presence of CO_2. After 1 h the spleen cells were decanted and cultured (B) (125%); the Mϕ sheet was washed and second set of DV-primed spleen cells were layered over it. After one hour the spleen cells were gently decanted and cultured (C), (17%). The remaining Mϕ cell sheet was washed and similarly incubated with third set of DV-primed spleen cells (D) (3%). DV-primed spleen cells were layered over normal Mϕ sheet for one h and then decanted and cultured (E), (−3%). After 24 h the PFC were counted and increase in help calculated as in Fig. 1.

spleen cell suspension and incubated at 37°C for 1 h in presence of 5% CO_2. These spleen cells were then gently decanted and cultured for 24 h at 37°C for PFC count. The Mϕ cell sheet was washed and layered over with the second set of DV-primed spleen cell suspension. After 1 h incubation at 37°C the cells were decanted and cultured. After repeating this cycle once more, the remaining Mϕ cell sheet was washed and harvested with the help of a rubber-tipped glass rod, and cultured with a fresh set of DV-primed spleen cell suspension. After 24 h DV-specific PFC was counted in the spleen cell cultures. The results in Fig. 5 show that in the first set of spleen cells exposed to HF-adsorbed Mϕ sheet the increase in help was 125%. The second set had the help reduced to 17% while in 3rd set it was only 3%. The helper activity was absent in the Mϕ collected after the third exposure.

Time required for transmission of helper signal from Mϕ

The results of the previous experiment suggested that the helper signal can be retrieved completely from Mϕ; therefore, an effort was made to determine the minimum exposure time required for the transmission of the signal. Multiple sets of HF-adsorbed Mϕ cell sheet were incubated with DV-primed spleen cells at 37°C in presence of 5% CO_2. At 10, 20, 30, 40 or 50 min of incubation the spleen cells of a set were separated from the Mϕ sheet by gently decanting and were cultured. After 24 h the helper activity was assayed by counting PFC. The data presented in Fig. 6 show that increase in help was 38% when DV-primed spleen cells were kept in contact with HF-adsorbed Mϕ for 10 min. The activity increased with the duration of exposure reaching a maximum of 174% after 40 min and was 172% after 50 min.

Cell to cell contact is necessary for the transmission of the helper signal

This experiment was done to investigate whether the helper signal is transmitted by

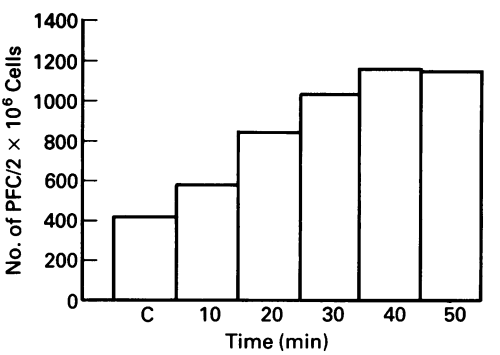

Fig. 6. Time required for transmission of helper signal from Mϕ. Multiple sets of HF-adsorbed Mϕ cell sheet were layered over with DV-primed spleen cells. At different periods (10 min, 38%; 20 min, 100%; 30 min, 145%; 40 min, 174%; 50 min. 172%) the spleen cells were decanted and cultured. C, DV-primed spleen cells as control (0% help). After 24 h the PFC were counted and increase in help calculated as in Fig. 1.

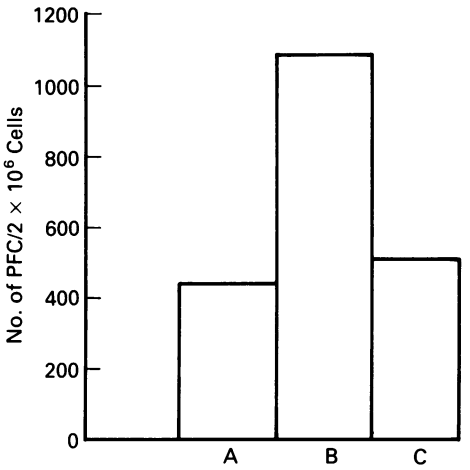

Fig. 7. Cell to cell contact of HF-adsorbed Mϕ and B cells is necessary for transmission of the helper signal. A, DV-primed spleen cells cultured as such for control (0% help); B, DV-primed spleen cell separated from the chamber containing HF through a cell-impermeable membrane (145%); C, DV-primed spleen cells separated from the chamber containing HF-adsorbed Mϕ through a cell impermeable membrane (15%). After 24 h the PFC were counted and increase in help calculated as in Fig. 1.

close contact of the cell membranes or via secretion of a soluble product. The HF-adsorbed Mϕ were separated from the DV-primed spleen cells by a cell impermeable Millipore membrane (pore size 0.45, Millipore Filter Corporation, Boston, Mass.) and were cultured for 24 h at 37°C in presence of 5% CO_2. After 24 h PFC were counted in spleen cells. For control, HF as such was separated from DV-primed spleen cells. The findings presented in Fig. 7 show that the PFC count in untreated DV-primed spleen cells was $443 \pm 30/2 \times 10^6$ while that in the experiment where HF was separated by a Millipore membrane from the cells was $1088 \pm 56/2 \times 10^6$, the increase in help being 145%. In contrast, when HF-adsorbed Mϕ were separated from DV-primed spleen cells the count was $510 \pm 43/2 \times 10^6$, the increase being 15%.

Direct interaction of TH with B cells

DV-induced TH enhance the clonal expansion of DV-specific B cells (Chaturvedi *et al*, 1985, 1987). The present experiment was done to investigate whether TH transmit the signal to B cells directly or only through secretory HF. For this, B cells (8×10^6 cells) enriched from DV-primed mouse spleen cells were mixed with TH (8×10^6 cells) and cultured for 24 h at 37°C in presence of 5% CO_2. In the second set, TH were separated from the B cells by a Millipore membrane and cultured. The third set consisted of TH mixed with DV-primed total spleen cells and in the fourth set HF was mixed with DV-primed total spleen cells. The controls consisted of DV-primed spleen cells or its enriched B cells cultured without TH. DV-specific IgM PFC were counted after 24 h of culture at 37°C. The findings presented in Fig. 8 show that the PFC counts in B cells (Control) were $448 \pm 28/2 \times 10^6$ cells without addition of TH. The counts were $432 \pm 36/2 \times 10^6$ when B cells were separated from TH by a Millipore membrane. On the other hand

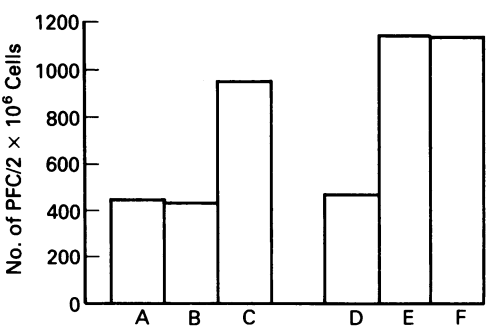

Fig. 8. Cell to cell contact of TH and B cell is necessary for transmission of helper signal. A, B cells enriched from DV-primed spleen cells were cultured as such for control (0% help); B, the B cells were separated from the chamber containing TH (−4%); C, the B cells were cultured with TH (111%). Total DV-primed spleen cells were D, cultured as such for control (0%); or E, were mixed with TH (144%); or F, inoculated with HF (142%). After 24 h the PFC were counted and increase in help calculated as in Fig. 1.

when TH and B cells were kept in close contact the counts were $945 \pm 65/2 \times 10^6$, the increase in help being 111%. Cultures containing total spleen cells mixed with TH had counts of $1137 \pm 29/2 \times 10^6$, the increase in help being 144%. Thus, TH transmitted helper signal to B cells by direct contact of the plasma membranes and the transmission was significantly higher ($P = \leqslant 0.001$) when Mϕ were also present.

Discussion

The most significant finding of the present study is that the DV-specific helper signal to the B cells can be transmitted only by a cell; either by the TH or, in its absence, by HF-adsorbed Mϕ; the B cells cannot accept soluble HF directly.

HF is composed of two polypeptide chains. The α- chain has antigen and the β-chain has H2-A determinants; the presence of both chains is essential for helper activity (Chaturvedi *et al*, 1991). The findings presented here show that both α and β-chains of HF were adsorbed on Mϕ and the presence of both chains was essential for the presentation of helper signal to the B cells.

It was observed that HF had no effect on B cells in absence of live Mϕ and the enhancement of PFC count occurred when Mϕ were present in the milieu. HF uptake was similar in both live and killed Mϕ but it could be presented to target B cells only by live Mϕ. Thus, HF uptake is a passive phenomenon but its presentation requires active cell metabolism. It was also observed that the adsorbed HF remained only on the surface of Mϕ and could be retrieved from them completely, and this process was completed in 40 min. How does HF remain tightly bound to the surface of Mϕ? We have shown that HF acts only in syngeneic (H-2^k haplotype) and not in other (H-2^d or H-2^q haplotypes) strains of mice (Chaturvedi *et al*, 1992). Of the two polypeptide chains of HF, one binds to the anti-H2-A^k antibody in an immunosorbent column (Chaturvedi *et al*, 1991). This chain also bound to the Mϕ, as shown here, thus providing active binding of HF. Similar results were also obtained with DV-induced suppressor factor (Chaturvedi *et al*, 1982; Shukla & Chaturvedi 1983).

Transmission of signal from one cell to another can be mediated either through secretion of a soluble mediator or by close contact of the plasma membranes of the interacting cells. The findings of the present study showed that the transmission of helper signal occurred by the close contact of the plasma membranes of TH-B cells or HF-adsorbed Mϕ-B cells. In another study we have shown physical binding of HF-pulsed Mϕ with ^{51}Cr-labelled B cells, which depended upon the dose of HF. The binding was specifically inhibited by anti-HF-antisera. HF also enhanced the binding of DV-pulsed Mϕ with the ^{51}Cr-labelled B cells (N. Rizvi, P. Chaturvedi *et al.*, unpublished manuscript). Further, the HF-adsorbed Mϕ neither release HF nor any other soluble product which may mediate enhancement of DV-specific IgM PFC. This conclusion has been drawn from the experiment in which HF-adsorbed Mϕ failed to transmit helper signal when separated from spleen cell cultures through cell-impermeable membranes. Similarly, TH separated from B cell enriched population through cell-impermeable membrane did not transmit the helper signal. The increase in PFC count by keeping TH in contact with enriched B cells was 111% and when Mϕ were also present in the milieu a significantly greater increase in help was observed ($P \leqslant 0.001$). A similar transmission of DV-induced suppressor signal from TS cells to TS2 cells via Mϕ has been reported (Chaturvedi *et al*, 1982; Shukla & Chaturvedi 1983).

A model is available of physical interaction of TH and B cells. Membrane proteins are involved in mediating antigen-specific physical interaction between T and B cells, which is H2 restricted. CD4 has been shown to play an essential role in TH-activation and in the TH-dependent triggering of B cells. Direct TH-B-cell contact is required for B cell activation; responding B cells must express

determinants for which TH are specific (reviewed by Noelle & Snow 1990). Inhibition studies with anti-class II mAbs indicate the essential function of H2 molecules in the physical interactions between antigen-specific T cell clones and antigen-specific, resting B cells (Sanders *et al*, 1986; Noelle *et al*, 1989). On the basis of these reports, Noelle and Snow (1990) have proposed that in the first phase, activation of TH occurs by H2-restricted interaction of antigen-specific conjugates and by the binding of intercellular adhesion molecule-1 (ICAM-1) to LFA-1, CD_4 to monomorphic domains of the class II proteins, and T cell receptor recognition of the class II-antigen complex. In the second phase, TH express novel proteins on their surface within 4–8 hours of activation which triggers B cell entry into the proliferation cycle.

The possible mode of action of HF here appears to bring DV antigen and H2-A molecules together to enhance the immune response (Chaturvedi *et al*, 1991). This could occur only with the active support of a cell membrane (TH or Mϕ) which focuses it in appropriate configuration and concentration on to the B cells. The relationship of HF with the cell-free form of TCR α,β-dimer that stimulates B cells (Guy *et al*, 1989) is not known, though they share a number of properties, as stated earlier. If in subsequent studies HF is shown to be a TCR, the present study will have the significance of presenting evidence that TCR too requires a cellular vehicle for its action. The question which arises is, If the TH can transmit the signal to B cells, what is the need to produce HF, which has the obligatory requirement of Mϕ? It has been suggested that the product of one T cell can activate about 100 Mϕ thus amplifying the signal (Waksman 1979). Thus, even with a lower frequency of TH, a significant number of B cells may be triggered with the help of HF and Mϕ.

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