

# Dengue virus-induced suppressor factor has two disulphide-bonded chains which bear anti-idiotypic and I-A and I-J determinants

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Dengue type 2 virus (DV) infection in mice induces generation of suppressor T cells which produce a soluble suppressor factor (SF). Activity of SF was abrogated by pretreatment with compounds like reduced glutathione, cysteine or 2-mercaptoethanol, which cleave disulphide bonds between polypeptide chains. Suppressor activity was restored when the chains of SF, separated by dithiothreitol treatment, were allowed to reassociate. This indicated need for the presence of both polypeptide chains in proper association. Experiments using immunosorbent columns showed that one chain had anti-idiotypic (DV) and I-A determinants while the other had I-J determinants. These determinants may impart antigen specificity and genetic restriction to the transmission of DV-induced suppressor signal.

THE murine immune response to dengue type 2 virus (DV) involves the generation of helper T cells and three distinct subpopulations of suppressor T cells<sup>1-3</sup>. DV induces generation of a subpopulation of splenic suppressor T cells (Ts<sub>1</sub>)<sup>4,5</sup>, which produce a soluble suppressor factor (SF)<sup>6</sup>. SF is taken up by macrophages both *in vitro* and *in vivo*<sup>7,8</sup> and is presented to naive T cells to recruit a second generation of suppressor T cells (Ts<sub>2</sub>), which produce a second suppressor factor (SF<sub>2</sub>)<sup>9</sup>. SF<sub>2</sub> recruits the third generation of suppressor T cells (Ts<sub>3</sub>)<sup>10</sup>, which mediate antigen-specific suppression of humoral immune response. Further Ts<sub>1</sub>, SF and Ts<sub>3</sub> bind anti-DV antibody while Ts<sub>2</sub> and SF<sub>2</sub> bind DV antigen—which result in abrogation of their activity—, indicating an idio-type-anti-idio-type-like network<sup>11</sup>.

The molecular structure of suppressor and helper factors induced by various synthetic antigens has been studied. These factors may consist of a single or two polypeptide chains<sup>12-16</sup>. DV-induced SF has been purified and characterized to some extent but its precise molecular structure is not known. SF is a low-molecular weight, heat-labile, acid and alkaline pH-labile, highly potent protein and acts in a dose-dependent manner<sup>2</sup>. SF is adsorbed by syngeneic macrophages<sup>7</sup>, and immunosorbent columns carrying anti-DV antibody<sup>11</sup>, and anti-I-J<sup>k</sup> and anti-I-A<sup>k</sup>, antibodies against gene products of the I region of the mouse major histocompatibility complex (MHC). There

is considerable interest in knowing the structure of anti-idiotypic T-cell suppressor factors. Here we show that DV (anti-idiotypic) and I-A determinants are present on one chain of SF and I-J determinants on the other.

## Methods

*Preparation of suppressor factor.* Three-to-four-month-old inbred Swiss albino mice from the colony maintained in the department were inoculated intracerebrally with 1000 LD<sub>50</sub> of DV. The virus, strain P23085, was in the form of brain suspension obtained from virus-infected adult mice<sup>18</sup>. After 8 to 11 days, the spleens were collected aseptically from the sick, moribund mice and homogenized in phosphate-buffered saline (PBS) to obtain SF<sup>6,9,19</sup>. It was distributed in small aliquots and stored at -20°C until use.

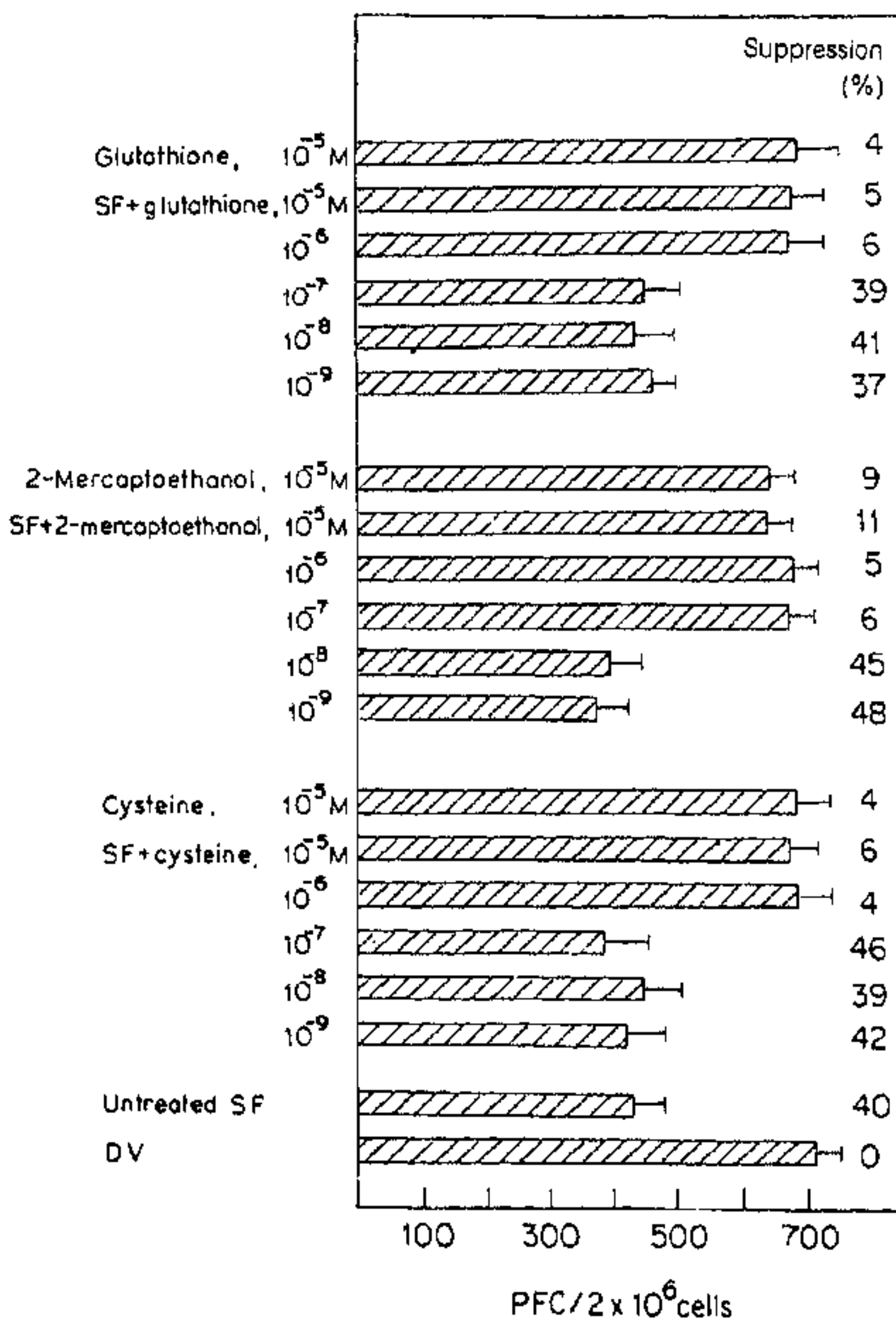
*Preparation of immunosorbent columns.* Immunosorbent columns of cyanogen bromide-activated Sepharose 4B (Pharmacia) were prepared by coupling anti-I-J<sup>k</sup>, anti-I-A<sup>k</sup> antibody (gift from Dr Marc Feldmann, London) or anti-DV antibody<sup>11</sup> as described by Theze *et al.*<sup>20</sup> and Waltenbaugh<sup>21</sup>. The columns were prepared in 3 ml syringes. Two mg of antibody protein was used per ml of packed gel. The columns were washed extensively with PBS and the wash fluid was monitored at 280 nm. The preparations of SF were adsorbed on these columns for 20 min at room temperature, the effluents were collected, the columns then washed with 10 ml of PBS and elution performed using 0.1 M glycine-HCl buffer, pH 2.5. The pH of the eluates was adjusted to 7.2 immediately. The effluent and eluate were assayed for suppressor activity.

*Assay of suppressor activity.* Suppressor activity of SF was determined in haemolytic plaque assays by the localized-haemolysis-in-gel technique of Jerne and Nordin<sup>22</sup> as described by Tandon and Chaturvedi<sup>23</sup>. Mice inoculated with a dose of 1000 LD<sub>50</sub> of DV intraperitoneally were inoculated *i.v.* with 0.2 ml of SF preparation 48 h later. Care was taken to ensure that the dilution factor remained constant in all SF preparations. Since peak PFC response is obtained on

days 6 and 7 post-inoculation, splenic DV-specific IgM antibody plaque-forming cells (PFC) were prepared on days 6 and 7 and used with DV antigen-coated sheep RBC as indicator<sup>18</sup>. Multiple slides were prepared for each mouse.

**Effect of treatment with reducing agents**

Glutathione, 2-mercaptoethanol and cysteine are agents that reduce disulphide linkages between polypeptide chains, thus cleaving them. Mice given untreated SF showed a 40% suppression of DV-specific IgM PFC response (PFC after DV alone 710±44, PFC after DV followed by SF 429±48; see Figure 1). SF treated with 10<sup>-5</sup> M or 10<sup>-6</sup> M glutathione caused only 5 or 6% suppression, indicating abrogation of SF activity. Treatment of SF with 10<sup>-7</sup>, 10<sup>-8</sup> or 10<sup>-9</sup> M glutathione did not affect suppressor activity. Similar results were obtained with 2-mercaptoethanol and cysteine; the former was effective up to 10<sup>-7</sup> M. The compounds themselves had no effect on the PFC response.



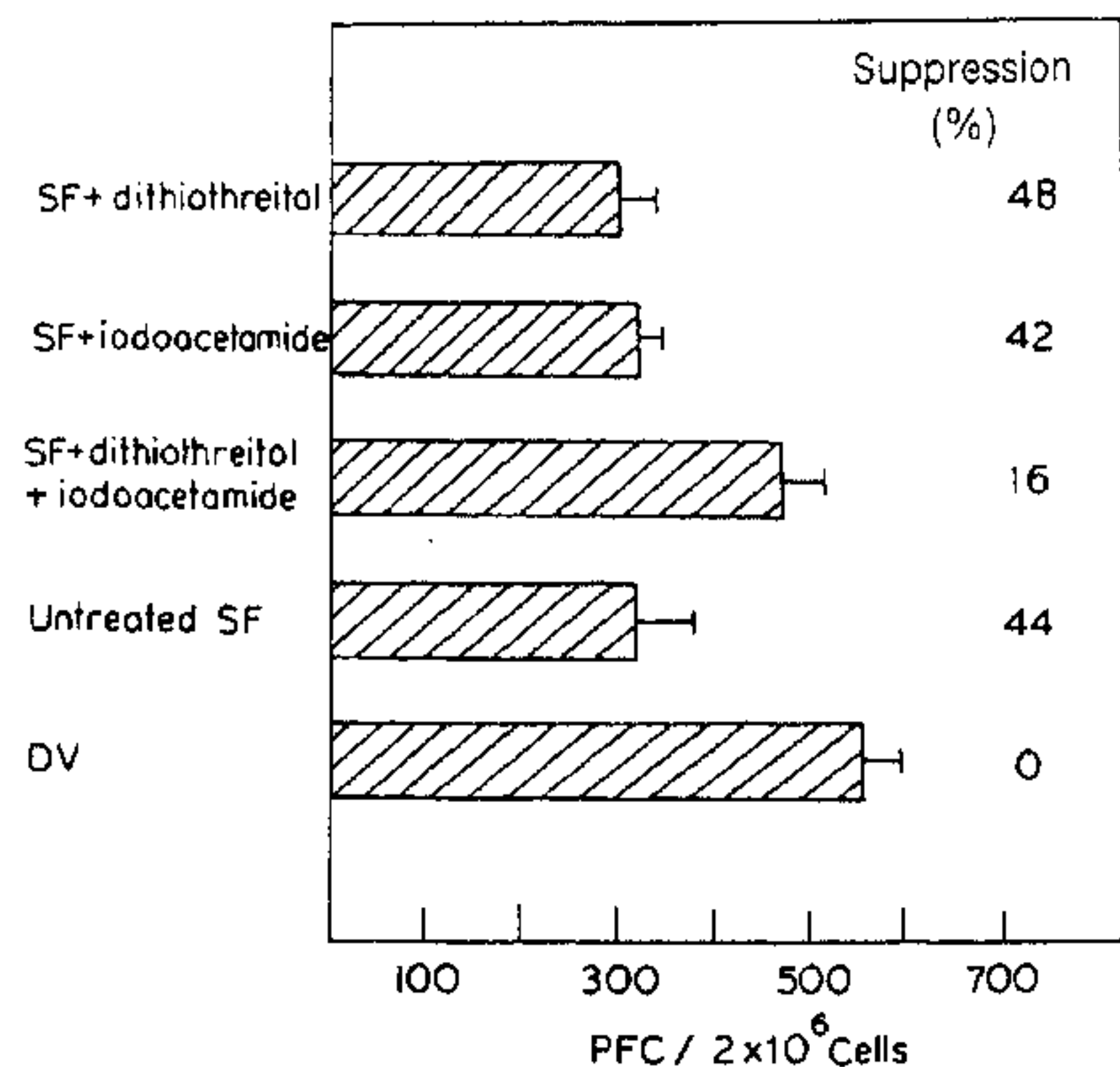
**Figure 1.** Abrogation of suppressor activity of SF by treatment with compounds that cleave disulphide bonds between polypeptide chains. DV, mice primed with DV and not given SF.

**Effect of treatment with dithiothreitol and iodoacetamide**

Dithiothreitol (DTT), in the presence of Tris-HCl buffer, reduces disulphide linkages between polypeptide chains and brings about their cleavage. Immediate treatment with iodoacetamide results in alkylation of the thiol groups and prevents reassociation of the reduced polypeptide chains<sup>12</sup>. SF treated with 5 mM DTT in the presence of 0.15 M Tris-HCl buffer for 45 min at room temperature was alkylated with 100 mM iodoacetamide for 20 min at room temperature<sup>12, 15</sup>. Other aliquots of SF were either reduced by DTT or alkylated with iodoacetamide under the same conditions. SF treated with DTT or iodoacetamide alone caused 48 or 42% suppression of the PFC response (Figure 2). When reduction was followed by alkylation, suppression was only 16%. The results indicate that SF is composed of two polypeptide chains joined by disulphide bonds and the presence of both chains in proper association is essential for mediation of suppression.

**Behaviour on immunosorbent columns**

It has been shown earlier that SF binds DV-specific antibody in an immunosorbent column<sup>11</sup>. We have now studied binding of the two chains of SF cleaved by reduction with DTT on anti-DV antibody immunosorbent columns. As shown in Figure 3a, there was little suppressor activity in both effluent and eluate from the anti-DV column (11% and 9% suppression respectively, compared to 41% by untreated SF). However, a mixture of effluent and eluate produced 26% suppres-



**Figure 2.** Abrogation of suppressor activity of SF by treatment with dithiothreitol and iodoacetamide.



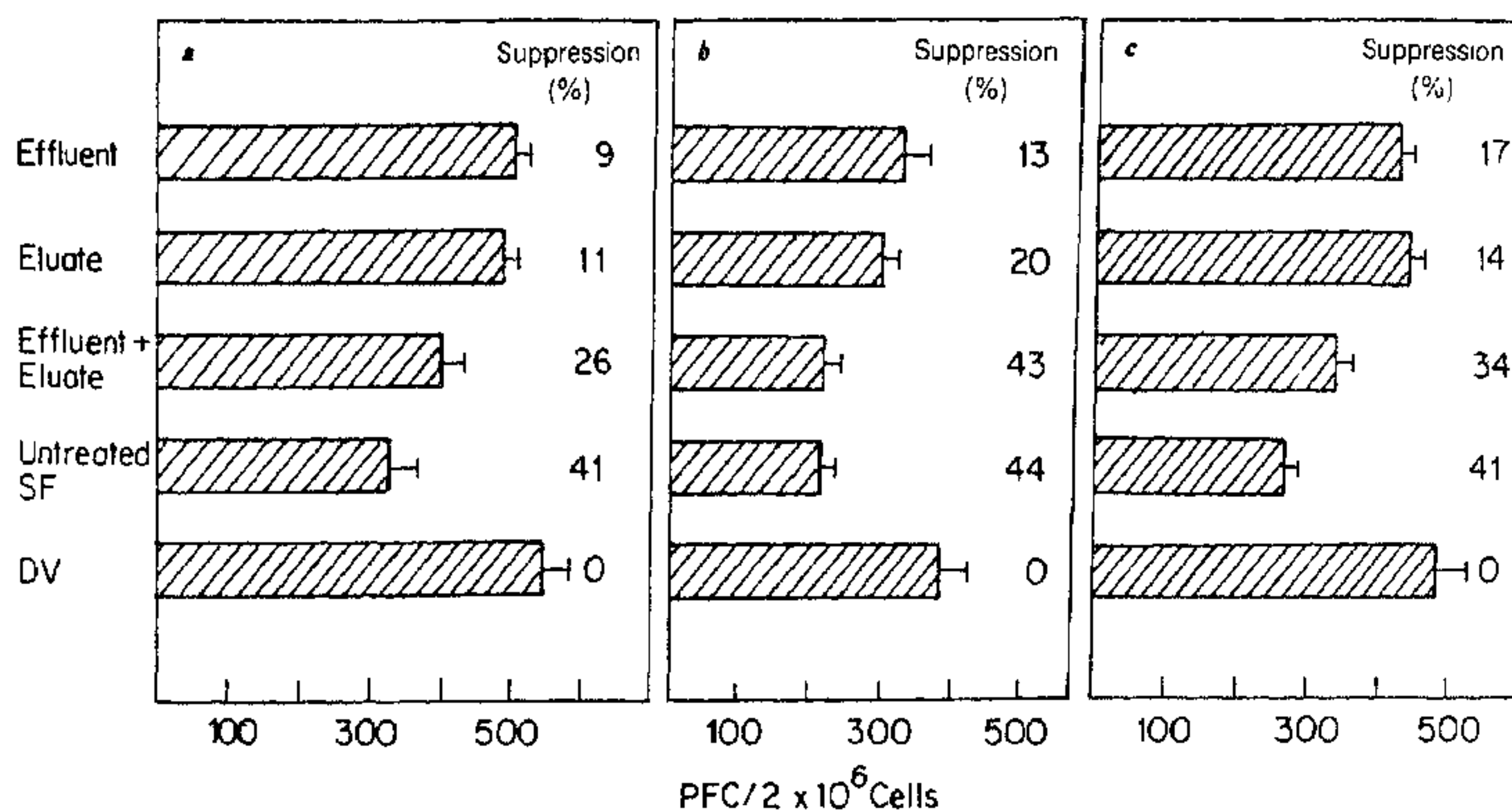


Figure 3. Suppressor activity of DTT-cleaved SF in effluent and eluate fractions after chromatography on (a) anti-DV, (b) anti-I-J and (c) anti-I-A immunosorbent columns.

sion. There was no binding on control columns prepared using normal sera in place of anti-DV antibody (data not shown) as the suppressor activity was present in the effluent.

Similarly, SF treated with DTT was run through anti-I-J and anti-I-A, immunosorbent columns, and effluent and eluate were assayed. In each case, full suppressor activity, not shown by either effluent or eluate alone, was restored when the two fractions were combined (Figure 3, b and c).

The results show that the chains of SF carry determinants for anti-DV antibody, and anti-I-J and anti-I-A antibodies. We then did mixing experiments to analyse the distribution of the three determinants on the two chains of SF. Eluates of SF from anti-DV, anti-I-J and anti-I-A columns were mixed in different combinations (Figure 4) and the suppressor activity of the mixtures was assayed. The mixture of eluates from anti-DV and anti-I-A columns had negligible suppressor activity (6%) whereas the mixture of eluates from anti-I-A and anti-I-J columns and the mixture of eluates from anti-DV and anti-I-J columns had significant suppressor activity (42 and 41% respectively, compared to 46% by untreated SF). We therefore conclude that the DV and I-A determinants are present on one chain of SF, while the I-J determinants are present on the second chain.

## Discussion

The suppressor activity of SF was abrogated, in a dose-dependent manner, by pretreatment with compounds

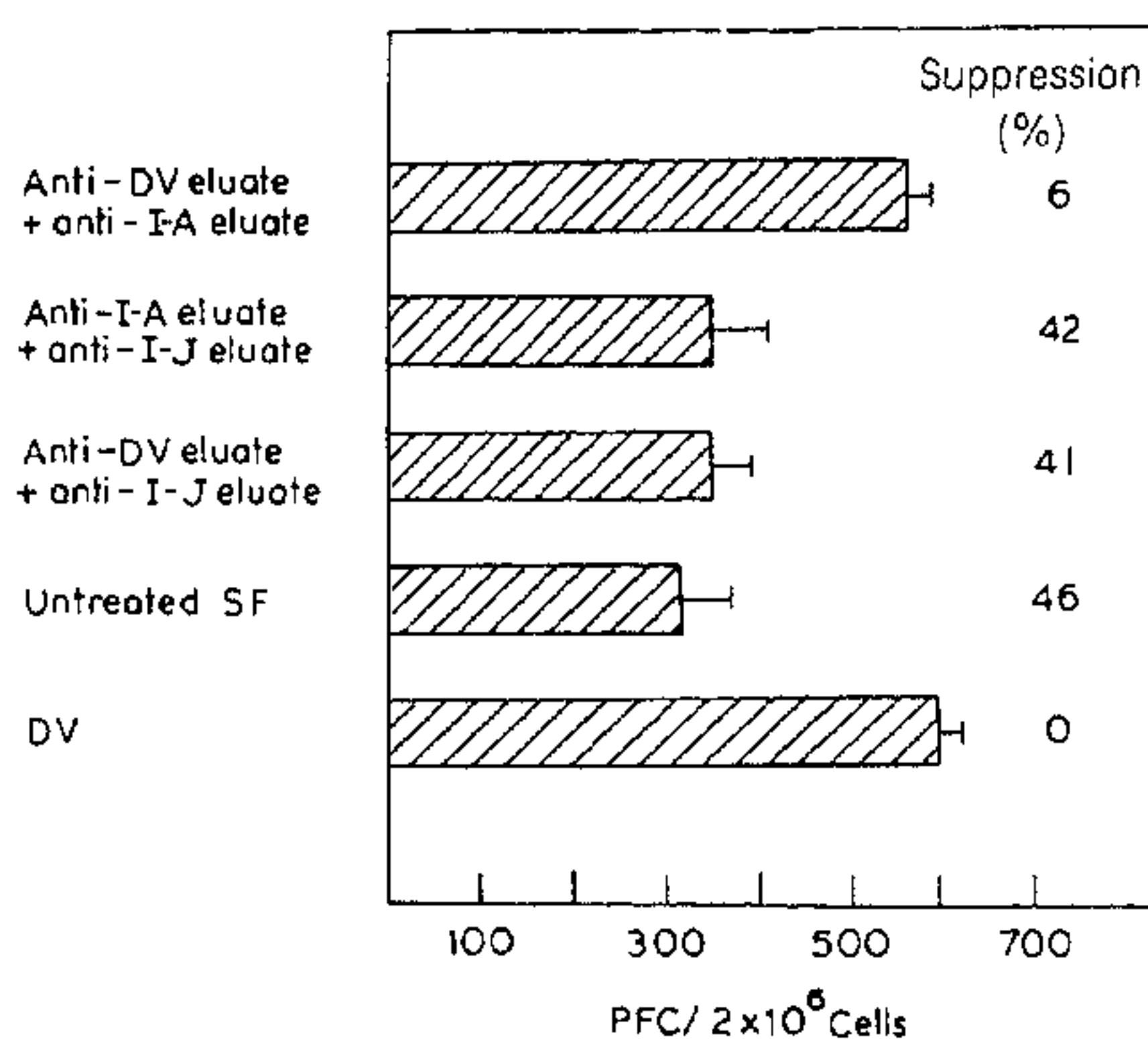


Figure 4. Suppressor activity of SF in mixtures of eluates of DTT-cleaved SF fractionated on anti-DV, anti-I-J and anti-I-A columns.

like reduced glutathione, cysteine and 2-mercaptoethanol, which are known to cleave disulphide linkages. The two dissociated polypeptide chains obtained after reduction of SF with DTT could reassociate, and suppressor activity was restored if reduced SF was not alkylated with iodoacetamide. This indicated the necessity for presence of both chains in proper association for mediation of suppression. Similar two-chain structures have been shown for suppressor factors induced by synthetic antigens<sup>12, 24</sup>, while others may have a single chain<sup>15</sup>.

In an earlier study, the presence of I-region specificities I-J<sup>k</sup> and I-A<sup>k</sup> was shown on SF<sup>17</sup>. The present study was aimed at locating the anti-idiotypic (DV) and the I-J and I-A determinants on the two chains of SF. Full suppressor activity was obtained in mixtures of anti-I-J column eluate and anti-I-A column or anti-DV column eluate. In contrast, the mixture of eluates from anti-DV and anti-I-A columns had no suppressor activity, showing that DV and I-A determinants are present on the same polypeptide chain. It has been reported that when the suppressor factor is a single-chain structure, the antigen and I-J determinants are present on the same chain; on the other hand, in a two-chain structure they are present on different chains<sup>15,25-27</sup>. Further, the 'antigen-positive' chain determines the specificity of the suppressor factor<sup>14</sup>.

The presence of I-A determinants on one chain of SF is interesting. The presence of I-J determinants is associated with suppressor function<sup>20,24,26</sup> while I-A are associated with helper functions<sup>16,28-30</sup>. On the other hand, Feldmann and Kontiainen<sup>31</sup> have summarized observations that indicate that I-subregion assignment of helper and suppressor factors need not necessarily be distinct and may not be a marker of function. Further, Marx<sup>32</sup> has quoted work that suggests that I-J suppressor factor contains modified forms of I-E or I-A molecules. Therefore the presence of both I-J and I-A determinants on DV-induced SF is possible. The finding that the chain with anti-idiotypic determinants also bears I-A is important in the light of the suggestion that I-J may be a recognition molecule for I-A.

Little *et al.*<sup>16</sup> suggested that the function of the two chains is to bring antigen and I-A molecules together on the cell surface. Absorption of helper factor by B cells has been suggested to be under I-A control<sup>29,30,33</sup>. SF induces generation of Ts<sub>2</sub> cells by transmitting the suppressor signal via macrophages; SF is adsorbed by syngeneic macrophages, which present it to the precursors of the Ts<sub>2</sub> cells<sup>7</sup>. Therefore it is likely that binding of SF to macrophages is under control of the polypeptide chain that bears I-A determinants; the same chain also bears DV determinants, which impart antigen specificity to the suppressor signal.

- Bendinelli, M. and Traka, Z.), Plenum Press, New York, 1988, p. 253.
3. Chaturvedi, U. C., Pahwa, M. and Mathur, A., *Indian J. Med. Res.*, 1987, **86**, 1.
  4. Tandon, P., Chaturvedi, U. C. and Mathur, A., *Immunology*, 1979, **38**, 653.
  5. Chaturvedi, U. C., Shukla, M. I. and Mathur, A., *Immunology*, 1981, **42**, 1.
  6. Chaturvedi, U. C. and Shukla, M. I., *Ann. Immunol. Pasteur Inst.*, 1981, **C132**, 245.
  7. Chaturvedi, U. C., Shukla, M. I. and Mathur, A., *Ann. Immunol. Pasteur Inst.*, 1982, **C133**, 83.
  8. Shukla, M. I. and Chaturvedi, U. C., *Br. J. Exp. Pathol.*, 1982, **63**, 522.
  9. Shukla, M. I. and Chaturvedi, U. C., *J. Gen. Virol.*, 1981, **56**, 241.
  10. Shukla, M. I. and Chaturvedi, U. C., *Br. J. Exp. Pathol.*, 1984, **65**, 267.
  11. Chaturvedi, U. C., Srivastava, N. and Mathur, A., *Curr. Sci.*, 1988, **57**, 691.
  12. Taniguchi, M., Saito, M., Takei, T. and Tokuhisa, T., *J. Exp. Med.*, 1981, **153**, 1672.
  13. Asherson, G. L., Colizzi, V., Zembala, M., James, B. M. B. and Watkins, M. C., *Cell Immunol.*, 1984, **86**, 448.
  14. Klyczek, K. K., Cantor, H. and Hayes, C. E., *J. Exp. Med.*, 1984, **159**, 1604.
  15. Jayaraman, S. and Bellone, C. J., *J. Immunol.*, 1985, **134**, 1010.
  16. Little, J. A., Asherson, G. L., Colizzi, V., James, B. M. B. and Hraba, T., *Immunology*, 1985, **55**, 713.
  17. Shukla, M. I. and Chaturvedi, U. C., *Br. J. Exp. Pathol.*, 1986, **67**, 563.
  18. Chaturvedi, U. C., Tandon, P. and Mathur, A., *J. Gen. Virol.*, 1977, **36**, 449.
  19. Shukla, M. I. and Chaturvedi, U. C., *Br. J. Exp. Pathol.*, 1984, **65**, 397.
  20. Theze, J., Kapp, J. A. and Benacerraf, B., *J. Exp. Med.*, 1977, **145**, 839.
  21. Waltenbaugh, C., *J. Exp. Med.*, 1981, **154**, 1570.
  22. Jerne, N. K. and Nordin, A. A., *Science*, 1963, **140**, 405.
  23. Tandon, P. and Chaturvedi, U. C., *Curr. Sci.*, 1977, **46**, 43.
  24. Asherson, G. L., Zembala, M., Colizzi, V. and Malkovsky, M., *Lymphokines*, 1983, **8**, 143.
  25. Kapp, J. A. and Araneo, B. A., *J. Immunol.*, 1982, **128**, 2447.
  26. Kapp, J. A., Sorensen, C. M. and Pierce, C. W., *J. Exp. Med.*, 1983, **158**, 1962.
  27. Krupen, K. *et al.*, *Proc. Natl. Acad. Sci., USA*, 1982, **79**, 1254.
  28. Taussig, M. J., Munro, A. J., Campbell, R., David, C. S. and Staines, N. A., *J. Exp. Med.*, 1975, **142**, 694.
  29. Lonai, P., Puri, J. and Briton, S., *J. Exp. Med.*, 1981, **154**, 942.
  30. Shiozawa, C., Sawada, S., Inazawa, M. and Diener, E., *J. Immunol.*, 1984, **132**, 1892.
  31. Feldman, M. and Kontiainen, S., *Lymphokines*, 1981, **2**, 87.
  32. Marx, J. L., *Science*, 1984, **223**, 573.
  33. Lonai, P., Puri, J. and Hammerling, G. J., *Lymphokines*, 1982, **5**, 197.

1. Chaturvedi, U. C., *Curr. Sci.*, 1984, **53**, 971.

2. Chaturvedi, U. C., in *Viral Immunosuppression* (eds. Friedman, H.,