Immunology 1984 **53** 491

Equivalence of conventional anti-picryl T suppressor factor in the contact sensitivity system and monoclonal anti-NP TsF_3: their final non-specific effect via the T acceptor cell

G. L. ASHERSON, M. E. DORF*, V. COLIZZI†, M. ZEMBALA‡ & BRIDGET M. B. JAMES
Division of Immunological Medicine, Clinical Research Centre, Watford Road, Harrow

Accepted for publication 27 June 1984

Summary. There is considerable confusion over whether the antigen-specific T suppressor factors (TsF) described by different authors are indeed equivalent. This paper investigates whether monoclonal TsF_3, obtained from hybridomas derived from mice injected subcutaneously with NP derived spleen cells, is functionally equivalent to the conventional T suppressor factor, produced by mice injected intravenously with chemically reactive, water soluble haptene (picrylsulphonic acid and oxazolone thioglycolic acid). Comparison of monoclonal anti-NP TsF_3 with conventional anti-picryl and anti-oxazolone T suppressor factor showed that both armed the non-specific T acceptor cell (Tacc) which was sensitive to cyclophosphamide and adult thymectomy. Moreover, non-specific inhibitor (nsINH) of the transfer of contact sensitivity was released when antigen, together with major histocompatibility complex products (MHC), reacted with conventional or monoclonal TsF on the surface of the non-specific T acceptor cell. The interaction of monoclonal TsF_3 with antigen, which led to the release of nsINH, required the presence of MHC and was I-J restricted. However, there was no Igh-1 restriction.

The equivalence of conventional anti-picryl and anti-oxazolone TsF has been demonstrated by arming the Tacc with a mixture of these two suppressor factors, and then triggering the release of nsINH with the mixed haptene 'picryl-oxazolone-lysine' which crosslinks separate molecules of TsF. A similar equivalence of conventional anti-oxazolone TsF and monoclonal anti-NP TsF_3 was demonstrated using the mixed hapten 'NP-oxazolone-lysine' to trigger the release of nsINH. It was concluded that monoclonal TsF_3 and conventional TsF were equivalent, and that both had an indirect mode of action through the non-specific T acceptor cell which led to the production of non-specific inhibitor.

INTRODUCTION

It is difficult to know the equivalence between apparently different T suppressor factors (TsF) because of the complexity of suppressor cell circuits and the fact that different authors use different antigens and different test systems. Moreoever, in the NP system, there is evidence for two distinct antigen-binding T

* Present address: Harvard Medical School, Department of Pathology, Boston, MA 02115, U.S.A.

† On leave from Istituto di Microbiologia, Facolta di Medicina e Chirurgia, Via S. Zeno 39, 56100 Pisa, Italy.

‡ Division of Clinical Immunology, Institute of Paediatrics, Copernicus Medical School, Wielicka 265, 30-663 Cracow, Poland.

Correspondence: Dr G. L. Asherson, Division of Immunological Medicine, Clinical Research Centre, Watford Road, Harrow HA1 3UJ.

suppressor factors, one (Ts_1) which induces the anti-idiotypic Ts_2 and the other (Ts_3) which blocks the expression stage of the contact sensitivity reaction. We therefore decided to take two relatively well-characterized systems and to compare the cells and factors at various points of the suppressor cell circuit. This comparison suggested that the Ts-eff in the picryl and oxazolone systems showed striking similarities to the Ts_3 cell in the NP (4-hydroxy-3-nitrophenylacetyl) system (Zembala *et al.*, 1982c; Sunday, Benacerraf & Dorf, 1981). In particular:

(i) both cells are induced by antigen presented in the context of I-J determinants (Colizzi, Asherson & James, 1983; Minami, Honji & Dorf, 1982b)

(ii) both cells are I-J$^+$ Lyt-2$^+$ (Sunday *et al.*, 1981; Malkovsky *et al.*, 1983)

(iii) both act at the effector stage of the delayed hypersensitivity skin reaction

(iv) both bind to antigen specifically (Zembala *et al.*, 1982b; Okuda *et al.*, 1981)

(v) both produce antigen-specific T suppressor factors which bear major histocompatibility complex (I-J) determinants; these bind to antigen and can be split by dithioerythritol (Okuda *et al.*, 1981; Sherr *et al.*, 1983; Asherson *et al.*, 1984b)

(vi) in both systems, the TsF show I-J genetic restriction in their action, although the test systems are different. In the picryl system, the TsF acts indirectly on the cell which transfers contact sensitivity. In fact, it arms the T acceptor cell. This cell lacks relevant intrinsic immunological specificity, but acquires it passively when armed with TsF. Subsequent interaction of antigen and MHC with the TsF on the surface of the Tacc leads to the release of a non-specific inhibitor (nsINH) of the transfer of contact sensitivity. This release of nsINH is I-J restricted (Zembala, Asherson & Colizzi, 1982a). Similary, the action of monoclonal TsF_3 in the NP system shows I-J genetic restriction, although the target for its action has not previously been identified (Minami, Furusawa & Dorf, 1982a).

This paper documents the equivalence between the monoclonal TsF_3 in the NP system (obtained from hybridomas derived from mice injected subcutaneously with NP derived spleen cells) and conventional TsF in the picryl and oxazolone system (produced by mice injected intravenously with chemically reactive, water soluble haptene), and shows that both act via the non-specific T acceptor cell (Tacc) to produce a non-specific inhibitor of the expression stage of the contact sensitivity reaction.

MATERIALS AND METHODS

Animals

The mice were bred at the CRC or purchased from OLAC Ltd. Mice aged between 8 and 12 weeks were used.

Suppressor factors

Conventional TsF was prepared from the spleen cells of mice injected with picrylsulphonic acid or 'oxazolone thioglycolic acid' and painted with picryl chloride or oxazolone (4-ethoxymethylene-2-phenyloxazolone) before harvesting. The 48 h supernatant of the spleen cells was used as a source of T suppressor factor (Zembala *et al.*, 1982a). Monoclonal anti-NP (4-hydroxy-3-nitrophenylacetyl) hybridoma TsF of CKB (H-2^k, Igh-I^b) and C57BL/6 (H-2^b, Igh-I^b) origin were prepared as described previously (Okuda *et al.*, 1981).

Antigens

'Bis-NP-lysine' was synthesized from 4-hydroxy-3-nitrophenylacetyl azide (NP azide) and lysine (Brownstone, Mitchison & Pitt-Rivers, 1966). The mixed haptene in which the alpha amino group of lysine was derived with NP, and the epsilon amino group with phenyloxazolone was prepared following the method for N$^\varepsilon$-picryl-L-lysine (Okuyama & Satake, 1980) followed by further derivation with NP azide. Bovine serum albumin and cells were haptenized with NP (Weinberger, Benacerraf & Dorf, 1980). However, half the volume of NP-N-succinimide was used, treatment was for 10 min and the glycylglycine and heparin were omitted. Cells were picrylated following Zembala *et al.* (1982c). Potassium chloride extracts containing MHC products were made following Reisfeld & Pellegrino (1971) and Asherson *et al.* (1984a).

Adult thymectomy and treatment with cyclophosphamide

Adult thymectomy was undertaken at 5 weeks and the mice were used at 8 weeks of age. Cyclophosphamide was given at 200 mg/kg 2 days before immunization.

Two-step T acceptor cell assay for antigen-specific T suppressor factor

This assay has two steps: firstly, the preparation of Tacc, their arming with TsF, the triggering of the release of non-specific inhibitor by antigen, together with MHC, and secondly, the assay of nsINH by the inhibition of the passive transfer of contact sensitivity.

The Tacc were nylon wool purified, 4-day oxazolone immune, BALB/c pooled lymph node and spleen cells. They were armed ($1{\cdot}5 \times 10^8$) with a total 4 ml of a single or mixture of conventional TsF and monoclonal TsF_3 for 1 hr at 37°. After dilution and centrifugation, they were triggered by adding a third of their number of haptenized cells in a volume of 5 ml, or by adding a total of 5 ml contaning 200 μg/ml final concentration of haptene (NP derivative of lysine or NP-albumin), together with potassium chloride extract (1 ml) derived from 5×10^7 normal spleen cells as a source of MHC. The supernatant after 2 hr was then used as nsINH.

NsINH was tested by incubating 4-day oxazolone immune passive transfer cells ($2{\cdot}5 \times 10^8$) in it for 45 min at 37°. The cells were diluted, washed, and $4–5 \times 10^7$ were injected into each of five recipients. The recipients were challenged immediately afterwards with 1% oxazolone in olive oil, and the increment of ear thickness was measured in units of 10^{-3}cm $\pm$ SD 24 hr later. Significance was assessed by Student's two-tailed *t*-test.

The positive control refers to mice which received immune cells incubated in 'dummy' supernatant, i.e. from T acceptor cells without either antigen or TsF. The negative control refers to mice which received no cells.

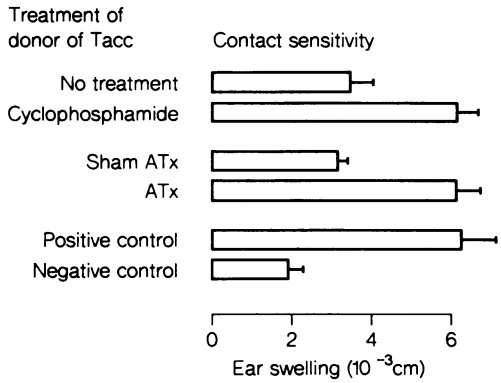

Figure 1. Monoclonal anti-NP TsF_3 acts via the T acceptor cell which is sensitive to cyclophosphamide and adult thymectomy. T acceptor cells were obtained from normal mice, or mice pretreated with 200 mg/kg cyclophosphamide 2 days before immunization, or sham or adult thymectomy (ATx) 3 weeks before immunization with oxazolone. The 4-day immune nylon T cells were then armed with anti-NP TsF_3 and triggered to release non-specific inhibitor by NP-derived CBA spleen cells. The non-specific inhibitor was assayed by its ability to inhibit the passive transfer of contact sensitivity; this is shown in units of 10^{-3} cm $\pm$ SD.

RESULTS

Monoclonal TsF acts via an adult thymectomy and cyclophosphamide sensitive cell

A distinctive feature of conventional TsF in the picryl system is that it fails to block the passive transfer of contact sensitivity by immune cells from mice pretreated with cyclophosphamide or subjected to adult thymectomy. Analysis showed that this was because TsF acted through a non-specific cyclophosphamide and adult thymectomy sensitive T acceptor cell (Zembala *et al.*, 1982c). The following experiment investigated whether monoclonal (mc) TsF in the NP system acted through a similar cell. In practice, Tacc were armed with monoclonal (mc) anti-NP TsF_3 and then triggered to release non-specific inhibitor by NP-derived spleen cells which served as a source of both antigen and MHC. The non-specific inhibitor was then tested for its ability to inhibit the passive transfer of contact sensitivity. Figure 1 (line 1) shows that the T acceptor cell population armed with TsF and then triggered with antigen, released non-specific inhibitor. However, no nsINH was produced when the donor of the T acceptor cell had received 200 mg/kg cyclophosphamide before immunization, or had been subjected to adult thymectomy.

MHC genetic restriction in the interaction of monoclonal TsF_3 and the haptenized cell used as a source of antigen

It is known that there is an I-J genetic restriction in the interaction of conventional TsF on the surface of the Tacc and the haptenized cell used as a source of antigen. Moreover, using monoclonal TsF_3, there is an I-J and an Igh-1 restriction between the factor and the recipient. The following experiment investigated the genetic restriction of mc TsF_3 in the T acceptor cell system. In the first block of Fig. 2, the Tacc was armed with mc TsF_3 of CKB (H-2^k, Igh-1^b) origin. The first two lines show that the release of nsINH was triggered by haptenized B10.BR cells which are syngeneic at both loci, and CBA cells which are syngeneic at the H-2 locus but not at Igh-1 with CKB. The third line shows that B10 mice which are H-2^b but share the same Igh-1^b failed to trigger the release of nsINH. These findings, taken together, indicate that triggering was H-2 but not Igh-1 restricted. The second block confirms these findings using mc TsF from B6 mice

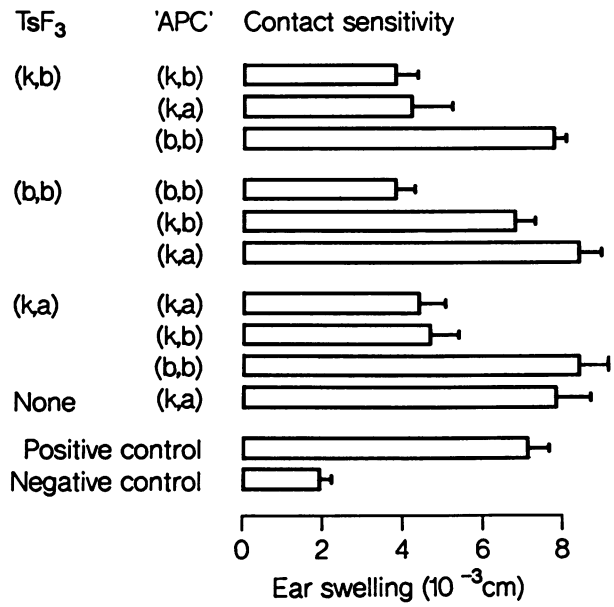

Figure 2. I-J but not Igh-1 genetic restriction in the interaction of monoclonal TsF_3 on the surface of the T acceptor cell with the NP-derived cell used as a source of antigen. In the first two blocks, T acceptor cells of BALB/c origin were armed with monoclonal TsF_3 from CKB (H-2^k, Igh-1^b) or B6 (H-2^b, Igh-1^b) origin. They were then triggered to release nsINH by NP-derived, B10.BR (H-2^k, Igh-1^b), CBA (H-2^k, Igh-1^a) or B10 (H-2^b, Igh-1^b) spleen cells ('antigen presenting cells' or 'APC'). In the third block, the Tacc was armed with conventional anti-picryl TsF from CBA mice and triggered with picrylated cells. The non-specific inhibitor was then assayed by its ability to inhibit the passive transfer of contact sensitivity. The H-2 and Igh-1 haplotypes of the TsF_3 and APC are shown in parentheses.

(H-2^b, Igh-1^b). Finally, the last block indicates that conventional anti-picryl TsF of CBA (H-2^k) origin had the same H-2 genetic restriction as mc TsF_3 of H-2^k origin.

The following experiment mapped the H-2 restriction more closely. T acceptor cells were armed with mc TsF_3 of CKB (H-2^k) origin and then triggered by antigen and potassium chloride extract containing MHC products. Figure 3 shows that antigen, together with B10.A(5R) (I-J^k) extract, triggered the release of nsINH, while 3R (I-J^b) extract was ineffective. As these two strains only differ at the I-J locus, it was concluded that there was an I-J genetic restriction. The remainder of the figure shows that extract of *k* haplotype (CBA) extract also caused triggering, while a *d* haplotype (BALB/c) extract was ineffective.

Equivalence of conventional and monoclonal T suppressor factor

It is known that the interaction of low molecular weight bivalent haptene with TsF on the surface of the Tacc leads to the release of nsINH (Asherson *et al.*, 1984a). The following experiment investigated whether this was also true for mc TsF_3. T acceptor cells were armed with mc TsF_3 and triggered by a mixture of bivalent haptene (bis-NP-lysine) and a source of I-J. The first line of Fig. 4 shows that this led to the release of nsINH. The second line provides a control and shows that no nsINH was released in the absence of the mc TsF_3.

It is also known that mixed haptene, e.g. picryl-oxazolone-lysine, triggers the release of nsINH from Tacc, providing they are armed with a mixture of anti-picryl and anti-oxazolone TsF. Under these circumstances, the two different T suppressor factors behave as though they are equivalent, and their crosslinking by the mixed haptene leads to triggering of the Tacc. The experiment also investigated whether conventional TsF and TsF_3 were similarly equivalent.

The third line of Fig. 4 shows that the mixed haptene NP-oxazolone-lysine triggered the release of nsINH when the Tacc was armed with a mixture of conventional anti-oxazolone TsF and mc TsF_3. The next two

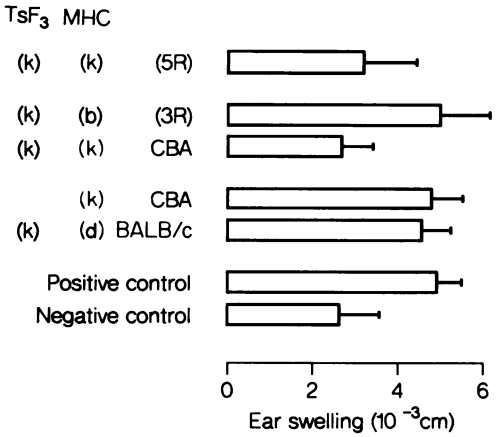

Figure 3. I-J genetic restriction in the interaction of monoclonal TsF_3 on the surface of the T acceptor cell, with antigen and potassium chloride extracts used as a source of MHC. T acceptor cells were armed with monoclonal TsF_3 from CKB (I-J^k) origin and then triggered to release nsINH by a mixture of NP-albumin and a potassium chloride extract of various cells used as a source of MHC. The I-J haplotypes of the TsF_3 and the extracts are shown in parentheses. The fourth line is a control illustrating that non-specific inhibitor is not produced unless the Tacc is armed with TsF_3. There is a significant difference ($P=0{\cdot}05$) between the inhibition seen when B10.A(3R) extract is used as a source of MHC and the lack of inhibition with B10.A(5R) extract. Similarly, there is a significant difference ($P=0{\cdot}01$) between the inhibition seen with CBA extract and the lack of inhibition with BALB/c extract.

lines provide controls and show that no triggering occurred when the Tacc were armed with only a single TsF. It was concluded that conventional and mc TsF_3 were functionally equivalent and triggered the Tacc when crosslinked to each other. Similar observations were made in two additional experiments.

DISCUSSION

These results extend the similarities described in the introduction between conventional TsF in the picryl and oxazolone contact sensitivity systems, and the monoclonal TsF_3 in the NP system.

(i) Both act through a non-specific T acceptor cell which generates non-specific inhibitor. This was already known for conventional TsF but had not been demonstrated for monoclonal TsF_3. In both cases, the T acceptor cell is sensitive to cyclophosphamide and adult thymectomy (Zembala *et al.*, 1982c). The observation that mc TsF_3 causes suppression when injected into immune mice treated with cyclophosphamide is apparently different from the present findings. However, in the present case, cyclophosphamide at 200 mg/kg was given 2 days before immunization to inactivate the T acceptor cells while, in the other case, 50 mg/kg was given 1 day after immunization to mice that subsequently received mc TsF_3 (Okuda *et al.*, 1981). In any case, TsF has an alternative mode of

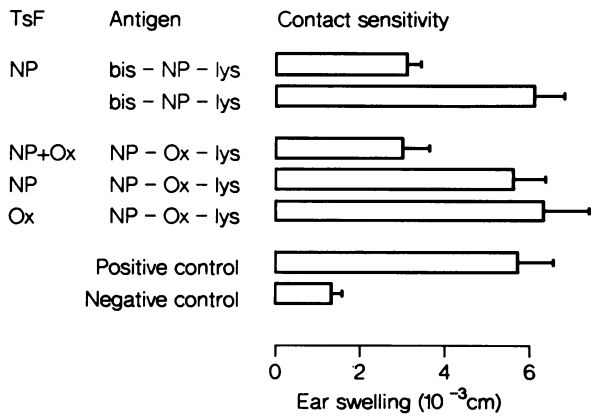

Figure 4. The triggering of the T acceptor cell cell armed with conventional anti-oxazolone and monoclonal anti-NP TsF_3 by the mixed haptene NP-oxazolone-lysine. T acceptor cells were armed with monoclonal TsF_3 from CKB mice or conventional anti-oxazolone TsF or with a mixture of the two. The release of nsINH was then triggered by the bivalent haptene bis-NP-lysine or by the mixed haptene NP-oxazolone-lysine. MHC was provided by potassium chloride extract of normal CBA spleen cells. The second line is a control illustrating that non-specific inhibitor is not released unless the Tacc is armed with TsF_3.

action via the macrophage (Ptak *et al.*, 1978), so that inactivation of T acceptor cells would not be expected to block its action completely *in vivo*.

(ii) In both cases, the interaction of antigen and MHC with the TsF on the surface of the Tacc is genetically restricted; this restriction maps to the I-J subregion.

(iii) Further evidence for the equivalence of monoclonal anti-NP TsF_3 and conventional anti-oxazolone TsF was provided by experiments in which the T acceptor cell was armed by both factors (Asherson *et al.*, 1984a). The release of non-specific inhibitor was then triggered by the mixed haptene NP-oxazolone-lysine which is univalent in respect to both haptenes. This was due to the crosslinking of molecules of the anti-oxazolone TsF to the anti-NP TsF_3. These findings illustrate the functional equivalence of these two suppressor facrors, and probably indicate that they attach to identical receptors on the T acceptor cell.

These results establish that the specific soluble T suppressor factors which have been characterized in the NP, picryl and oxazolone systems have identical functional properties in terms of their ability to arm the T acceptor cell. These properties are consistent with other data, demonstrating that these antigen-specific molecules have similar properties, including I-J determinants, antigen binding sites, susceptibility to dithioerythritol and action at the expression stage of the immune response.

The present results indicate in the NP system, where three distinct T suppressor cells (Ts_1, Ts_2, Ts_3) have been defined, that at least one more cell is involved in the suppressor circuit, namely the non-specific T acceptor cell. Furthermore, the I-J restriction previously ascribed to the TsF_3 is probably explained by the genetic restriction in the triggering of the armed T acceptor cell for which the critical requirement is matching in the I-J subregion between the source of the TsF and the MHC used in the triggering stage (Zembala *et al.*, 1982a).

There is an interesting difference between the genetic restrictions when TsF_3 is injected into immune recipients, and the genetic restriction when TsF_3 is used to arm the T acceptor cell. In the former case, there is an I-J and Igh-1 genetic restriction, and in the latter only an I-J restriction. Igh-1 genetic restrictions are usually interpreted as being due to idiotype anti-idiotype reactions, and the simplest explanation is based on the view that anti-idiotypic factors and antigen may be regarded as equivalent in the network theory and, hence, that anti-idiotypic factor can substitute for antigen. It is possible that, in the T acceptor cell system where haptenized cells are added as a source of antigen, there is no need for additional anti-idiotypic reactions, while when TsF_3 is injected *in vivo*, the amount of antigen may be limiting and anti-idiotype reactions then become important.

These experiments confirm the antigen specificity of TsF_3, while emphasizing that the final suppression is antigen non-specific. Our previous observations in the NP system failed to reveal a non-specific final event (Okuda *et al.*, 1981). Thus, in the present experiments, non-specific inhibitor was produced *in vitro* in the presence of high concentrations of antigen, while in the *in vivo* experiments, TsF_3 was injected into mice sensitive to dinitrofluorobenzene (DNFB) and the mice challenged with DNFB only. It is possible that, under these circumstances, anti-idiotypic factors which would only occur in NP-immunized mice would be necessary to activate non-specific suppression.

We conclude that the final phases of the suppressor pathway are common in the NP and picryl systems, and involve the non-specific T acceptor cell. Whether the initial phases are also similar is unresolved. Although it is clear that Ts_1 and Ts_2 form an integral part of the NP suppressor cell circuit, it is likely that other methods exist for triggering the Ts_3 cells. In particular, anti-idiotypic Ts_2 is not required when there is sufficient antigen to trigger the Ts_3. In keeping with this view, recent experiments show that mice primed with picrylsulphonic acid (but not painted with picryl chloride) which fail to produce active 'complete' TsF, release TsF when triggered with picrylated normal cells *in vitro* (V. Colizzi, M. Zembala and G. L. Asherson, unpublished observations).

ACKNOWLEDGMENTS

V. Colizzi and M. Zembala thank the European Molecular Biology Organization (EMBO) and the Wellcome Trust for financial support. M. Zembala was also supported by Grant No. 10.5 of the Polish Academy of Sciences.

REFERENCES

ASHERSON G.L., COLIZZI V., ZEMBALA M., JAMES B.M.B. & WATKINS M.C. (1984a) Nonspecific inhibitor of contact sensitivity made by T acceptor cells. Triggering of T cells armed with antigen specific T suppressor factor (TsF) requires both occupancy of the major histocompatibility complex recognition site by soluble I-J product and

crosslinking of the antigen recognition sites of the TsF. *Cell Immunol.* **83,** 389.

ASHERSON G.L., WATKINS M.C., ZEMBALA M. & COLIZZI V. (1984b) Subunit structure of T suppressor factor. Antigen specific T suppressor factor occurs as a single molecule and as separate antigen binding and $I\text{-}J^+$ parts both of which are required for biological activity. *Cell Immunol.* **86,** 448.

BROWNSTONE A., MITCHISON N.A. & PITT-RIVERS R. (1966) Chemical and serological studies with an iodine-containing synthetic immunological determinant 4-hydroxy-3-iodo-5-nitrophenylacetic acid (NIP) and related compounds. *Immunology*, **10,** 465.

COLIZZI V., ASHERSON G.L. & JAMES B.M.B. (1983) The role of I-J in the suppressor T-cell circuit which influences the effector stage of contact sensitivity: antigen together with syngeneic I-J region determinants induces and activates T suppressor cells. *Immunology*, **49,** 191.

MALKOVSKY M., ASHERSON G., CHANDLER P., COLIZZI V., WATKINS M.C. & ZEMBALA M. (1983) Nonspecific inhibitor of DNA synthesis elaborated by T acceptor cells. I. Specific hapten- and I-J-driven liberation of an inhibitor of cell proliferation by $Lyt\text{-}1^-2^+$ cyclophosphamide-sensitive T acceptor cells armed with a product of $Lyt\text{-}1^+2^+$-specific suppressor cells. *J. Immunol.* **130,** 785.

MINAMI M., FURUSAWA S. & DORF M.E. (1982a) I-J restrictions on the activation and interaction of parental and F1-derived Ts_3 suppressor cells. *J. exp. Med.* **156,** 465.

MINAMI M., HONJI N. & DORF M.E. (1982b) Mechanism responsible for the induction of I-J restrictions on Ts_3 suppressor cells. *J. exp. Med.* **156,** 1502.

OKUDA K., MINAMI M., FURUSAWA S. & DORF M.E. (1981) Analysis of T cell hybridomas. II. Comparisons among three distinct types of monoclonal suppressor factors. *J. exp. Med.* **154,** 1838.

OKUYAMA T. & SATAKE K. (1960) The preparation and properties of 2,4,6-trinitrophenyl aminoacids and peptides. *J. Biol. (Tokyo)*, **47,** 454.

PTAK W., ZEMBALA M., HANCZAKOWSKA-RECKLICKA M. & ASHERSON G.L. (1978) Nonspecific macrophage suppressor factor: its role in the inhibition of contact sensitivity to picryl chloride by specific T suppressor factor. *Eur. J. Immunol.* **8,** 645.

REISFELD R.A. & PELLEGRINO M.A. (1971) Salt extraction of soluble HL-A antigens. *Science*, **172,** 1134.

SHERR D.H., MINAMI M., OKUDA K. & DORF M.E. (1983) Analysis of T cell hybridomas. III. Distinctions between two types of hapten-specific suppressor factors that affect plaque-forming cell responses. *J. exp. Med.* **157,** 515.

SUNDAY M.E., BENACERRAF B. & DORF M.E. (1981) Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VIII. Suppressor cell pathways in cutaneous sensitivity responses. *J. exp. Med.* **153,** 811.

WEINBERGER J.Z., BENACERRAF B. & DORF M.E. (1980) Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. III. Interaction of effector suppressor T cells is restricted by H-2-Igh-V genes. *J. exp. Med.* **151,** 1413.

ZEMBALA M., ASHERSON G.L. & COLIZZI V. (1982a) Hapten-specific T suppressor factor recognizes both hapten and I-J region products on haptenized spleen cells. *Nature (Lond.)*, **297,** 411.

ZEMBALA M., ASHERSON G.L., COLIZZI V. & WATKINS M.C. (1982b) Desensitization *in vitro*: the role of T-suppressor cells, T-suppressor factor and T-acceptor cells in the inhibition of the passive transfer of contact sensitivity to picryl chloride by exposure to antigen *in vitro*. *Immunology*, **47,** 605.

ZEMBALA M., ASHERSON G.L., JAMES B.M.B., STEIN V. & WATKINS M.C. (1982c) Anti-haptene T suppressor factor acts through an $I\text{-}J^+$, Lyl^-2^+, T acceptor cell that release a nonspecific inhibitor of the transfer of contact sensitivity when exposed to antigen. *J. Immunol.* **129,** 1823.