A limitation in the conventional assay of interferon in serum or other biological fluids has been the need to dilute the sample until the effects of other non-interferon substances that affect viral growth no longer mask the action of the interferon. Dilution of a sample of lymphoblastoid interferon in either phosphate-buffered saline (PBS), 10% horse serum, 0.1% NaN_3 or in undiluted normal human serum resulted in identical titration curves (Fig. 3), indicating that the immunoradiometric assay overcomes this problem.

The lower limit in the measurement of serum interferon by biological assays has been sufficient to detect interferon in the serum of some patients with acute viral infections and in the serum of patients to whom interferon has been administered. This sensitivity is insufficient, however, to measure the interferon (if any) in normal human serum.

It should be possible to improve the sensitivity of the immunoradiometric assay still further by optimizing the conditions at low interferon concentrations. In any case the sensitivity of the assay can be further increased by prior concentration and purification of the interferon on a small NK2-affinity column.

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Evidence that the serological determinant of H–Y antigen is carbohydrate

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The histocompatibility-Y (H–Y) antigen is a minor histocompatibility antigen which has been detected on cell surfaces from the heterogametic sexes of mammalian, bird, amphibian, teleost and invertebrate species[1–3]. H–Y is thought to be a male-determining substance in mammals because of its almost perfect correlation with maleness among a variety of mammalian species. To characterize the molecular determinant responsible for H–Y-specific serological activity, H–Y-positive immunoabsorbent cells were first subjected to various treatments which alter protein or carbohydrate structure and then tested for their ability to absorb H–Y antisera. We present here evidence that the serological determinant of H–Y antigen is carbohydrate.

To assess the dependence of H–Y serological activity on polypeptide structure, absorbing cells were treated with the proteases trypsin, α-chymotrypsin and thermolysin. After enzyme treatment and washing, treated splenocytes were used to absorb an aliquot of H–Y antiserum. In this and all subsequent experiments, absorbed sera were tested for cytotoxic activity against male murine epidermal cells. Splenocytes treated with either trypsin, α-chymotrypsin or thermolysin showed almost the same absorptive capacities as untreated splenocytes (Fig. 1). These results indicate that H–Y-specific serological activity is resistant to these three proteolytic enzyme treatments.

To determine the dependence of H–Y serological activity on carbohydrates, absorbing cells were treated with periodate, which oxidizes 1,2-dihydroxyethyl linkages found in glycosyl residues. Splenocytes were treated with varying concentrations of periodate and washed in a protein + sugar-supplemented medium before absorption. Sensitivity of H–Y-specific absorption to periodate treatment, as well as subsequent experimental treatments, were quantified as corrected per cent inhibition of absorption, as defined in Fig. 2 legend. As a control, the same treatments were performed on absorbing cells bearing antigens of the $H\text{-}2^b$ haplotype. Such serological determinants are thought to be contained in amino acid sequences[4,5]. These data show H–Y-specific absorption to be ~ fivefold more periodate sensitive than H–2 (Fig. 2*a*).

A more direct assessment of the carbohydrate dependence of H–Y serological activity can be made by treating absorbing cells

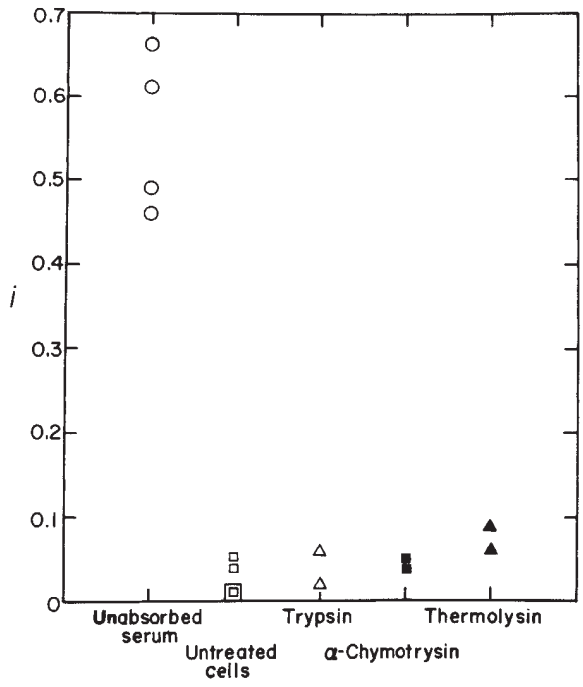

Fig. 1 H–Y-specific absorption: resistance to proteolytic enzymes. Purified trypsin (Gibco), α-chymotrypsin (Worthington), and thermolysin (Calbiochem) were dissolved in Puck's saline F (PSF) to yield individual suspensions containing 0.1% enzyme by weight. Samples containing 4×10^7 splenocytes were incubated in a 2-ml enzyme suspension at *p*H 7.4, 37 °C for 20 min, washed three times at 4 °C in Medium 199 containing 10% heat-inactivated (56 °C, 30 min) γ-globulin-free fetal calf serum (FCS, Gibco) and used to absorb 25-μl aliquots of rat H–Y antiserum diluted 1:4 in Medium 199 containing 10% FCS. Rat H–Y antiserum was prepared by the method of Fellous *et al.*[9] by immunizing female Fisher rats with male (Fisher) splenocytes. Before use in this experiment, rat antisera were heat inactivated (56 °C, 30 min) and absorbed with equal volumes of female murine splenocytes for 1 h at both 0 and 37 °C. An antiserum was regarded as H–Y specific if it had cytotoxic activity against male, but not female, C57BL/6J (B6) epidermal cells, and this activity could be absorbed by male, but not female B6 splenocytes. H–Y-specific immunoabsorption was done on ice (~0 °C) for 1 h. Absorbed sera were tested for direct cytotoxicity against B6 epidermal cells according to the method of Scheid *et al.*[10] with the exception that heat-inactivated γ-globulin-free FCS was used as a protein supplement for the isolation medium. Cell killing was quantified by the cytotoxic index, *i*, defined as: no. dead cells in sample − no. cells killed by complement dose/total no. cells − no. cells killed by complement alone.

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with exo-glycosylhydrolases which remove a terminal glycosyl residue from the non-reducing end of a carbohydrate chain. Splenocytes were treated with highly purified β-glucuronidase and β-galactosidase, then washed in protein-enriched media before absorption. Figure 2*b* shows a graph of corrected per cent inhibition of absorption versus units of enzyme used for treatment. Whereas H-Y-specific absorption is resistant to β-glucuronidase treatment (1,000 units of enzyme destroy 2% of activity from the absorbing cells), it is sensitive to β-galactosidase to the extent that 1,000 units of enzyme remove 54% of the H-Y absorptive capacity of male mouse splenocytes. To confirm that the inhibition by β-galactosidase is not due to uptake of the enzyme by the absorbing cells, the 1,000-unit enzyme treatment was repeated for H-Y in the presence of 0.1 M mannose-6-phosphate, which inhibits the uptake of β-galactosidase by mammalian cells. In the presence of mannose-6-phosphate, the 1,000-unit β-galactosidase treatment still destroyed 45% of H-Y-specific absorptive activity (Table 1). As another control, the 1,000-unit β-galactosidase treatment was used on absorbing cells specific for H-2^b antigens. H-2^b-specific absorption is completely insensitive to the action of 1,000 units of β-galactosidase (Table 1).

These data suggest that the integrity of an H-Y serological determinant is perturbed by the removal of a terminal galactose from the non-reducing end of an associated carbohydrate moiety. To test further the serological dependence of H-Y on a non-reducing terminal galactose residue, we performed a series of absorbing cell treatments using galactose oxidase, which oxidizes the terminal galactose residue at the non-reducing ends of a carbohydrate chain. Although galactose oxidase has no appreciable effect on H-2^b-specific absorption, it inhibits H-Y-specific absorption by male mouse splenocytes by as much as 38% in this experiment (Fig. 2*c*).

To verify that the inhibition of H-Y observed here is due to the specific oxidation of a terminal non-reducing galactose rather than to nonspecific oxidation at the cell surface, a similar experiment was carried out using glucose oxidase. Whereas 10 units of galactose oxidase inhibit H-Y-specific absorption by 38%, an equivalent amount of glucose oxidase had only an 8% inhibitory effect (Table 1). This value could possibly be due to

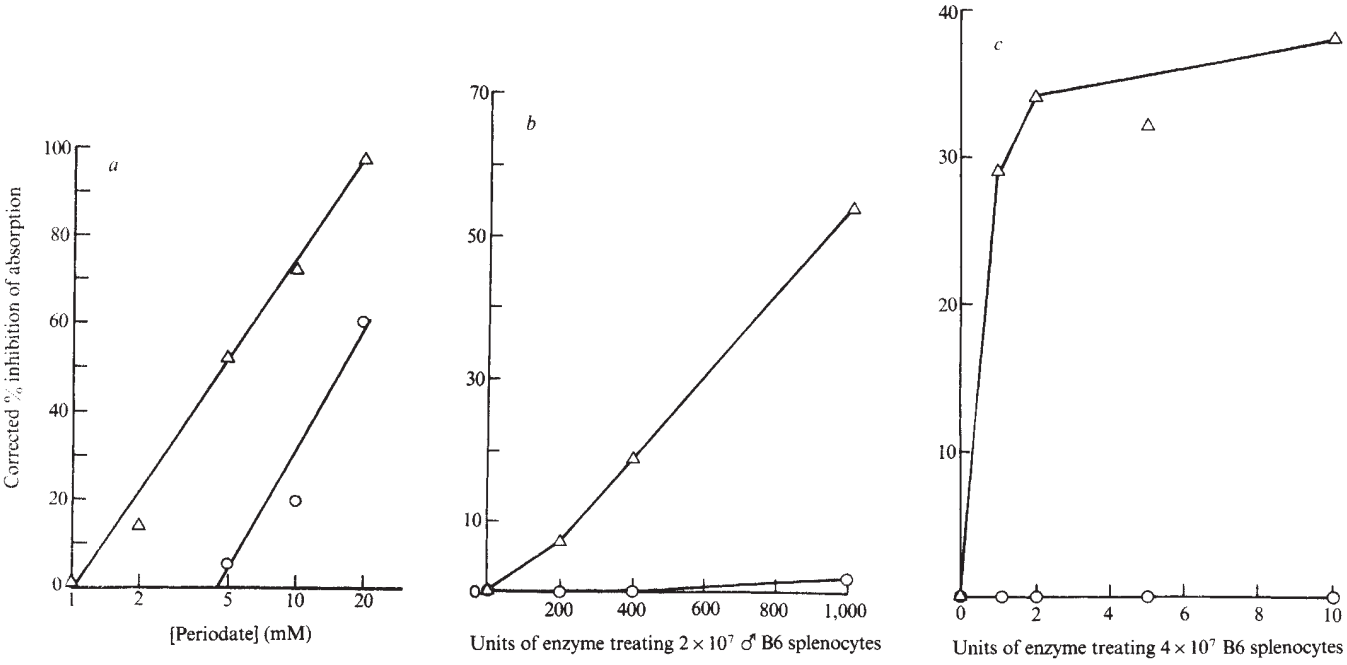

Fig. 2 Sensitivity of H-Y-specific absorption to all treatments quantified as corrected per cent inhibition of absorption, defined as:

$$\frac{i\,(\text{treated cells}) - i(\text{untreated cells})}{i(\text{unabsorbed serum}) - i(\text{untreated cells})}$$

with i defined as in Fig. 1 legend. Each per cent inhibition value represents the mean of duplicate samples. *a*, Known concentrations of periodic acid (Frederick Smith Chemicals) were dissolved in isotonic phosphate buffer and adjusted to *p*H 7.4. Splenocytes were treated at a concentration of 10^7 cells per ml (10^7 total cells for H-2 and 2×10^7 total cells for H-Y) for 10 min at room temperature (~22 °C). After treatment, the cells were washed twice in Medium 199 containing 10% FCS at 4 °C. Treated splenocytes were used to absorb either mouse H-Y (diluted 1:4) or H-2^b antiserum (diluted 1:100) as described in Fig. 1 legend. Mouse H-Y antiserum was prepared as described by Krco and Goldberg[4]. H-2^b antiserum was an equivolume mixture of alloantisera to murine H-2.2(D-2) and H-2.33(D-33) specificities obtained from the NIH. Absorbed H-Y antiserum was tested for cytotoxicity as described above. H-2^b antisera were tested for direct cytotoxicity against splenocytes in the following way. Equal amounts (10 μl) of H-2^b antiserum and B6 splenocytes (8×10^6 per ml) were incubated for 20 min at room temperature; 10 μl of baby rabbit complement (Pel Freez) diluted 1:4 was then added and the suspension incubated at 37 °C for 35 min. Trypan blue (10 μl of 0.2% dye) was added for the final 5 min of incubation to stain dead splenocytes. Formalin saline (10 μl containing 5% formaldehyde) was added as a fixative and live and dead cells were counted. ○, H-2^b; Δ, H-Y. *b*, Highly purified rat β-glucuronidase (○) and bovine β-galactosidase (△) (both 12,000 units per mg protein) were obtained from Dr Virginia Hieber, Department of Pediatrics and Communicable Diseases, University of Michigan Medical School. Splenocytes (2×10^7 total) were treated with known amounts of enzyme in 2.0 ml incubation volumes containing phosphate buffered saline (PBS) at *p*H 6.8, 37 °C for 1 h. After treatment, cells were washed twice in Medium 199 containing 10% FCS at 4 °C before absorption. Antisera used for absorption and subsequent cytotoxicity testing were murine H-2^b and rat H-Y antisera as described above. *c*, Galactose oxidase (Sigma) was dissolved in PBS containing 1.0 mg per ml of bovine serum albumin (BSA, Sigma). Splenocytes were incubated with known amounts of enzyme in PBS, *p*H 7.4, at 37 °C for 1 h. Each incubation mixture contained 10^7 (in the case of H-2^b, ○) or 4×10^7 (in the case of H-Y, △) splenocytes plus enzyme at a volume of 0.5 ml. After incubation, treated cells were washed in Medium 199 supplemented with 1.0 M galactose before absorption. Antisera were either murine H-2^b or rat H-Y as described above. Absorption and subsequent cytotoxicity testing were as before.

Table 1 Specificities of enzyme treatment

Enzyme treatment	Total enzyme (U)	% Inhibition of absorption (mean ± s.e.) H–2[b]	H–Y
β-Galactosidase alone	1,000	0 ± 1	54 ± 8
β-Galactosidase + 0.1 M mannose-6-phosphate	1,000	–	45 ± 4
Galactose oxidase	10	0 ± 1	38 ± 2
Glucose oxidase	10	0 ± 1	8 ± 2

β-Galactosidase treatments in the presence of mannose-6-phosphate (Sigma) were as described for Fig. 2*b* with the exception that 0.1 M mannose-6-phosphate was added to the treatment buffer. Glucose oxidase treatments were as described for Fig. 2*c* with the exceptions that glucose oxidase (Sigma) was the enzyme used and that treated cells were washed in Medium 199 supplemented with 1.0 M glucose.

the presence of contaminating amounts of galactose oxidase in our enzyme preparation (2% according to manufacturer). In addition, H–2[b] was tested for inhibition of absorption by glucose oxidase and no effect was observed (Table 1). These data are consistent with the suggestion that the integrity of an H–Y serological determinant is perturbed by structural alterations directed against a terminal non-reducing galactose on an associated carbohydrate moiety.

The serological reactivity of H–Y antigen, as measured by immunoabsorption, is demonstrated here to be resistant to the action of various proteolytic enzyme treatments. This result implies that the H–Y serological determinant is not a protein, or that it is part of a polypeptide chain resistant to proteolysis. The resistance to proteolysis of H–Y, in combination with its greater sensitivity to periodate than H–2 suggest that the H–Y-specific serological determinant is more likely to be carbohydrate than protein. In most of the experiments reported here, H–2 is used as a control as it is a well characterized glycoprotein antigen whose serological activity is resistant to treatments directed at carbohydrate structures, but sensitive to protein-directed treatments[5,6].

We have reported that H–Y-specific absorption is sensitive to both β-galactosidase and galactose oxidase treatments. The sensitivity of H–Y-specific absorption to both these enzymes is in the range 29–54%. Although these values do not reflect complete inhibition, they are consistent with the accepted model of antigen–antibody interaction involving binding of the combining site of an antibody molecule with a carbohydrate determinant of 4–6 glycosyl residues[7]. By perturbing a terminal galactose residue on such a carbohydrate determinant, one would expect detectable, although not complete, inhibition of antibody binding. Thus, our observations of β-galactosidase and galactose oxidase sensitivity indicate a role for a terminal non-reducing galactose residue in the serological integrity of H–Y antigen.

If the serologically active portion of H–Y is carbohydrate, this could account for its evolutionary conservation, suggested by the broad serological cross-reactivity of H–Y-like substances from a variety of species[1,2,8]. A carbohydrate nature for this determinant could also explain why antisera raised against it are of low titre (1:16–1:32 in the case of our antisera) and affinity. Although the results reported here suggest a carbohydrate nature for the H–Y serological determinant, direct demonstration of whether this immunological determinant is itself a carbohydrate, or is strictly dependent on a carbohydrate chain requires further experimentation.

This work was presented at the meeting of the American Society for Cell Biology in Cincinnati, Ohio on 14–18 November 1980. The abstract of this presentation is published elsewhere[8].

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Fibrinolysis with acyl-enzymes: a new approach to thrombolytic therapy

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Deep vein thrombosis in man presents a considerable clinical challenge. Despite the availability of prophylactic measures, therapeutic thrombolysis is often necessary, but is difficult and hazardous. Treatments have included the administration of plasmin, other less specific proteolytic enzymes, the indirect plasminogen activator, streptokinase, and the direct activators, urokinase and streptokinase–human plasmin complex. All these treatments have been associated with some haemostatic breakdown, which has discouraged their widespread application. The enzyme components of the coagulation and fibrinolytic pathways can, in general, be classed as serine proteases[1], with a catalytic mechanism which operates via acyl-enzyme intermediates[2]. Chase and Shaw[3] showed that *p*-nitrophenyl-*p'*-guanidinobenzoate could specifically acylate the active centre of trypsin-like enzymes, giving rise to a stable *p*-guanidinobenzoyl enzyme and other stable acyl-enzymes have since been described[4,5]. We report here the fibrinolytic use of acylated derivatives of plasmin (E.C.3.4.21.7) and streptokinase–plasmin(ogen) complexes.

An acyl-plasmin, catalytically inert and therefore unable either to degrade systemic plasma proteins or react irreversibly with its principal plasma inhibitors (α2-antiplasmin[6,7] and α2-macroglobulin[8]), may still bind to a fibrin clot because the lysine/fibrin binding sites on the 'kringle' domains of plasmin are structurally and functionally separate from the catalytic centre[9] (Fig. 1). Deacylation may then occur to give fibrin-bound plasmin. Wiman and Collen[10] have shown that the involvement of the lysine binding sites of plasmin in binding to fibrin reduces the effectiveness of α2-antiplasmin and provides part of the molecular basis for selective fibrinolysis *in vivo*[11]. Thus, if an acyl-plasmin is administered to an animal, the release of plasmin by deacylation of fibrin-bound acyl-plasmin would be expected (within certain kinetic limits) to lead to fibrinolysis. In the systemic circulation, however, α2-antiplasmin would be expected to act rapidly to neutralize the plasmin released by deacylation, provided that the rate and dose were not so great as to consume the α2-antiplasmin content of the plasma. The concept of active centre acylation may also be applied to plasminogen activators, provided that they too possess fibrin affinity independent of the catalytic centre. In that case, acylation would allow the activator to reduce interactions with plasma plasminogen which lead, during current therapy in man, to harmful plasminaemia[12]. The putative advantages of therapeutic thrombolysis with acyl-plasmins and acyl-activators, therefore, are (1) enhanced efficacy, due to evasion of inhibitor systems; (2) sustained-release pharmacokinetics, leading to simplified administration and easier clinical control; (3) reduction of

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