Research Article

Recombinant Human Interferon Alpha-2a: Delivery to Lymphoid Tissue by Selected Modes of Application

Andreas Supersaxo,[1,4] Wayne Hein,[2] Harald Gallati,[3] and Hans Steffen[1]

Received November 13, 1987; accepted February 5, 1988

The effect of different parenteral administration routes (i.d., s.c., i.v.), infusion rates, and albumin contents of the drug vehicle on the cumulative recovery of recombinant human interferon alpha-2a (rIFN alpha-2a) in lymph and on its concentration in blood and lymph was determined in sheep. Blood samples were withdrawn from a jugular vein catheter and lymph was collected from a cannulated efferent popliteal lymphatic duct. The concentration of rIFN alpha-2a in lymph and blood plasma samples was measured by an enzyme immunoassay. Following i.v. infusion of 2×10^7 U of rIFN alpha-2a, the peak concentrations measured in blood plasma and lymph, respectively, were 8250 and 14 U/ml. The concentration measured in lymph after i.d. or s.c. administration of the same dose was about 10^5 times higher (peak concentration, 3.1×10^6 U/ml), while blood plasma levels remained low (peak concentration, 315 U/ml). The cumulative recovery of rIFN alpha-2a in lymph following i.d. or s.c. administration was $59.2 \pm 7\%$ (mean $\pm$ SD; $N = 8$) and was affected neither by the infusion rate nor by the coadministration of albumin. Our data indicate that following i.d. or s.c. administration, rIFN alpha-2a (MW 19,000) is absorbed mainly by the lymphatics. This results in high levels of rIFN alpha-2a in the lymph which drains from the application site to the regional lymph nodes. The knowledge gained in this investigation may help to improve the mode of administration and therapeutic efficacy of protein drugs whose targets are lymphoid cells.

KEY WORDS: recombinant human interferon alpha-2a (rIFN alpha-2a); parenteral administration; lymph concentration; blood plasma concentration; cumulative lymph recovery; sheep.

INTRODUCTION

Interferons are proteins which can be used as antiviral, cytostatic, or immunomodulatory agents. Based mainly on empirical approaches, several routes and schedules of administration have been used, but neither an optimal route nor an optimal dosage schedule is yet at hand. This is particularly true when interferons are used as immunomodulatory drugs (1).

Interferons can be considered as paracrine hormones acting physiologically in microenvironments, rather than as circulating proteins (2). Thus in order to reproduce the physiological distribution of interferon (3), Bocci has proposed that lymphatic absorption of interferons should be facilitated (4).

So far only a few studies have examined the lymphatic delivery of interferons. Yoshikawa *et al.* measured the concentration of interferon in the thoracic duct of rats following enteral administration. They demonstrated that the administration of interferon with mixed micelles resulted in a highly selective delivery into the lymphatics, but the total amount absorbed was very small (~0.05%) (5–8). Bocci *et al.* studied the lymphatic absorption of interferon following parenteral administration in rabbits. They showed that after subcutaneous injection, the total amount of interferon recovered in thoracic duct lymph was less than 0.04%. Recoveries increased from two- to eightfold when interferon was injected with albumin or with hyaluronidase, respectively (9).

To our knowledge no studies have examined the concentration of interferon in the peripheral lymphatic system following parenteral administration. In the present work we investigated the influence of different parenteral administration routes (i.d., s.c., i.v.), infusion rates, and albumin contents of the drug vehicle on the absorption of recombinant human interferon alpha-2a (rIFN alpha-2a) by lymphatic and blood vascular routes. The experiments were done in sheep after the efferent lymphatic duct of the popliteal lymph node had been cannulated. The results obtained indicate that following i.d. or s.c. administration, rIFN alpha-2a (MW 19,000) is absorbed mainly by the lymphatics. This results in high levels of rIFN alpha-2a in the lymph which drains to the regional lymph nodes.

MATERIALS AND METHODS

Interferon Preparation

Human serum albumin-free lyophilized rIFN alpha-2a

[1] Pharmaceutical Research/Preclinical Development, F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.
[2] Basel Institute for Immunology, Basel, Switzerland.
[3] Central Research Units, F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.
[4] To whom correspondence should be addressed.

0724-8741/88/0800-0472$06.00/0 © 1988 Plenum Publishing Corporation

containing 18 × 10^6 U per vial (Ro 22-8181/658, Lot No. G FER 13 031, F. Hoffmann-La Roche & Co.) was used. The amount of rIFN alpha-2a is expressed in international units (U) of antiviral activity determined in the vesicular stomatitis virus MDBK cell assay. The specific activity of the pure unformulated substance was 2–4 × 10^8 U/mg protein (protein purity of >98%). Details of the products and purification procedures are described elsewhere (10).

Animals

Nonpregnant, nonlactating white Alpine or black Jura ewes or wethers (50–70 kg) were obtained from Versuchsbetrieb Sennweid, Olsberg, Switzerland. Prior to experiments animals were housed in pens inside a barn with access to pasture in spring and summer. During experiments sheep were maintained in individual pens and fed on commercial pellets.

Surgical Procedures

All surgical procedures were done under aseptic conditions in a properly equipped operating theater. Surgical anesthesia was induced by intravenous injection of sodium thiopentone and maintained with halothane (Hoechst, Frankfurt am Main, FRG). The efferent duct of the popliteal lymph node was cannulated using the procedure described by Hall and Morris (11). Catheters were inserted into the jugular vein through the bore of a 14-gauge needle and anchored in position with stay sutures.

rIFN Alpha-2a Absorption Studies

The rIFN alpha-2a absorption studies were started 2–3 days after surgery. The general design of our absorption studies is shown in Fig. 1. After collecting blank blood and lymph samples, 2 ml of a rIFN alpha-2a solution (1 × 10^7 U/ml) was injected either i.d. or s.c. into the lower part of the hind leg or i.v. into the jugular vein. In some experiments human serum albumin was coadministered.

The s.c. and i.d. administrations were done with an injection device attached to the shaved leg. This device allows accurate injection of drug solutions into a defined place within the skin. The depth of injection was 2.25 mm for the s.c. administration and 1.25 mm for the i.d. administration. The infusion rate was controlled by an infusion pump (Micropump MP-20, Micrel, Greece) and was varied from 0.3 to 17 ml/hr. Lymph was collected continuously in heparinized tubes. The collection tubes were changed at 10-min intervals for the first hour after injection, at hourly intervals for the next 5–6 hr, and then at 4- to 12-hr intervals. The volume of each lymph sample was determined gravimetrically using a specific gravity of 1.0. Blood samples were withdrawn from a jugular vein catheter and transferred to heparinized tubes. The blood samples were taken at 15-min intervals for the first hour after injection and then hourly for the next 4 hr. Between periods of sampling the cannula was kept patent with a heparin lock (50 U/ml in saline). Care was taken to obtain a circulating blood sample by discarding the first 3 ml of aspirate. After centrifugation (2000 rpm, 20 min, 4°C) all lymph and blood plasma samples were kept frozen at −20°C until assayed.

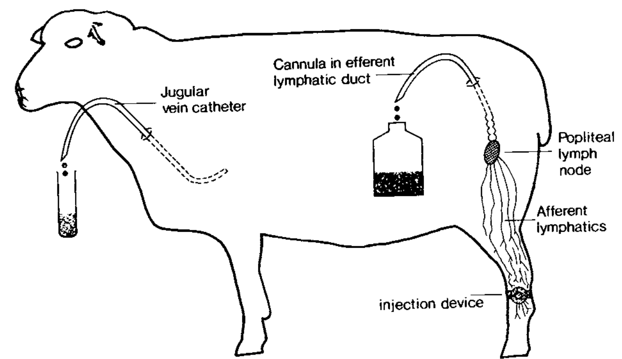

Fig. 1. General design of absorption studies.

rIFN Alpha-2a Assay

The concentration of rIFN alpha-2a was measured by an enzyme immunoassay method, utilizing a solid-phase sandwich principle (12). Briefly, rIFN alpha-2a was incubated with (mouse) monoclonal rIFN alpha-2a antibodies (LI-9) that were coated on a polystyrene bead and with (mouse) monoclonal rIFN alpha-2a antibody (LI-1) that was conjugated to horseradish peroxidase. Following this incubation step, the unbound material was removed by washing and the activity of peroxidase bound to the bead was measured using *O*-phenylenediamine as substrate. The resulting color intensity, which is measured photometrically, is directly proportional to the rIFN alpha-2a concentration in the sample. The reference standard had a specific activity of 1.7 × 10^8 U/mg of protein as determined against the NIH interferon standard. The assay sensitivity in lymph and blood plasma was 1 U/ml of rIFN alpha-2a. The concentration of rIFN alpha-2a measured with the enzyme immunoassay has been correlated with antiviral activity in the vesicular stomatitis virus MDBK cell assay (13).

RESULTS

Cumulative Recovery of rIFN Alpha-2a in Lymph

The effect of the administration route, infusion rate, and albumin content of the drug vehicle on the cumulative recovery of rIFN alpha-2a in the efferent popliteal lymph was determined. The recovery of rIFN alpha-2a in lymph was calculated as the product of the concentration in lymph and the volume of lymph and was expressed as a percentage of the administered dose.

The results of 12 experiments performed in six separate sheep are summarized in Table I. The cumulative recovery of rIFN alpha-2a in lymph following i.d. or s.c. administration into the lymph cannulated leg was 59.2 ± 7% (mean ± SD; $N = 8$) and was affected neither by the infusion rate nor by the coadministration of albumin. In contrast, following i.v. infusion or i.d. administration into the noncannulated leg, only a small amount of the administered dose was recovered in the lymph. The cumulative recoveries determined were 0.0015 ± 0.0007 and 0.006 ± 0.002%, respectively ($N = 2$).

Table I. Effect of Administration Route, Infusion Rate, and Albumin Content of Drug Vehicle on Cumulative Recovery of rIFN alpha-2a in the Efferent Popliteal Lymph

Sheep No.	rIFN alpha-2a injection[a] Route	Duration	Albumin conc. (%)	Recovery in the efferent popliteal lymph (% dose)
3836	i.d.	20 min	0	65.2
3670		20 min	0	65.7
3667		20 min	0.5	54.2
3568		20 min	13	53.6
3118		6 hr	0	64.7
3670		6 hr	0	53.3
3836	s.c.	7 min	0	50.1
3568		20 min	0	63.8
3836	i.d.[b]	7 min	0	0.008
3670		20 min	0	0.004
3670	i.v.	20 min	0	0.001
3570		20 min	0.5	0.003

[a] rIFN alpha-2a, 2×10^7 U, was dissolved in 2 ml of drug vehicle and administered as shown.
[b] Injected into opposite, noncannulated leg.

Concentration of rIFN Alpha-2a in Lymph and Blood Plasma

Whereas the high recovery of rIFN alpha-2a in the lymph following i.d. or s.c. administration was the most important result, we were nevertheless interested in the timecourse–concentration profiles in lymph and plasma. Typical concentration curves of rIFN alpha-2a in the efferent popliteal lymph and in blood plasma following i.v. and i.d. administration are shown in Figs. 2 and 3. The data presented in Fig. 2 are those obtained following the administration of 2×10^7 U in a human serum albumin-free solution as a 20-min infusion. The lymph concentration–time data (Fig. 2a) show that i.d. administration of rIFN alpha-2a into the lymph cannulated leg results in very high lymph levels. The concentration of rIFN alpha-2a reached a peak of 3.1×10^6 U/ml after 20 min. In contrast, the peak concentrations in lymph following either i.v. infusion or i.d. administration into the noncannulated leg were about 10^5 times lower than after i.d. administration into the lymph cannulated leg. Maximum concentrations of 14 and 60 U/ml, respectively, were determined. The corresponding concentrations of rIFN alpha-2a in blood plasma are shown in Fig. 2b. Following i.v. infusion the rIFN alpha-2a peak concentration measured at the end of a 20-min infusion was 8250 U/ml. The maximum concentrations determined following i.d. administration into the cannulated or noncannulated leg were 38 and 315 U/ml, respectively.

Figure 3 shows the concentration profiles and cumulative recoveries of rIFN alpha-2a in the efferent popliteal lymph following i.d. administration of 2×10^7 U in a human serum albumin-free solution as a 20-min or 6-hr infusion. After the 20-min infusion the rIFN alpha-2a concentration reached a peak value of 3.1×10^6 U/ml and decreased rapidly thereafter (Fig. 3a). Following the 6-hr infusion the rIFN alpha-2a level reached a plateau concentration of about 2×10^5 U/ml after 1 hr. This concentration remained nearly constant during the whole infusion time and decreased slowly after the end of the infusion (Fig. 3b).

The concentration–time data shown in Figs. 2 and 3 are comparable with those obtained from the experiments in which either the depth of injection, the albumin content of the drug vehicle, or the infusion rate was varied. The following minor differences were observed. Compared with the 20-min infusion, the concentrations of rIFN alpha-2a measured in lymph following i.d. or s.c. administration as a 7-min infusion peaked earlier, at 10 min, and were slightly higher. The maximum measured concentrations were 3.5×10^6 and 4×10^6 U/ml, respectively. On the other hand, the coadministration of albumin resulted in a delayed appearance of rIFN alpha-2a in lymph. The concentration of rIFN alpha-2a in lymph following i.d. application in a 13% albumin solution over 20 min reached a peak of 2.5×10^6 U/ml after 1 hr.

DISCUSSION

In the present investigation we measured the concentration of rIFN alpha-2a in blood plasma and lymph following different administration routes, infusion rates, and

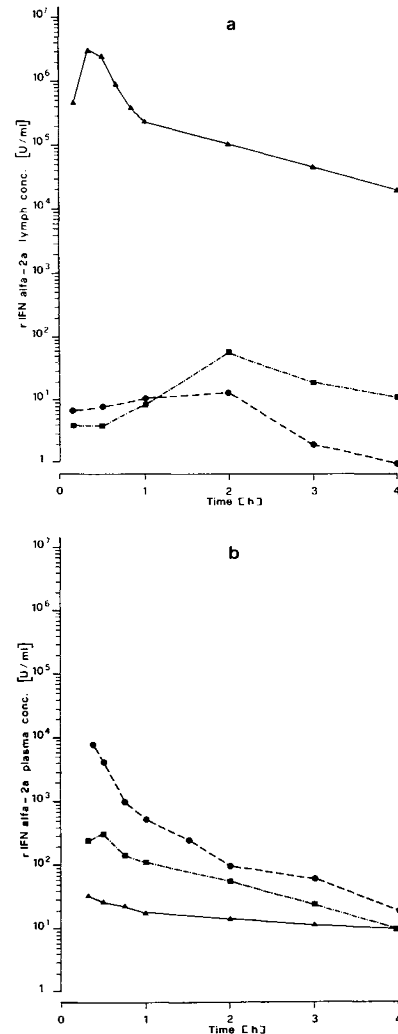

Fig. 2. Concentration of rIFN alpha-2a in efferent lymph from the right popliteal lymph node (a) and in blood plasma (b) following parenteral administration of 20×10^6 U: (●) i.v. (jugular vein); (■) i.d. (left, noncannulated leg); (▲) i.d. (right, cannulated leg).

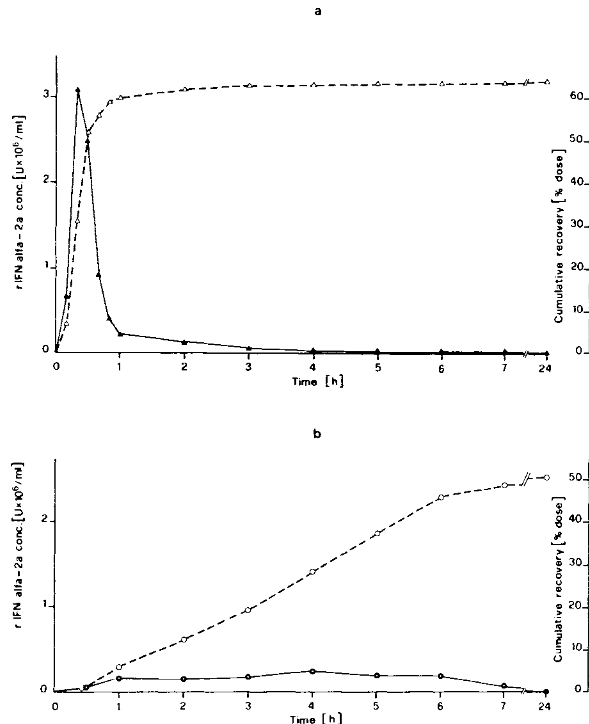

Fig. 3. Concentration profiles and cumulative recoveries of rIFN alpha-2a in efferent lymph from the right popliteal lymph node after i.d. administration of 20×10^6 U into the right hind leg as a 20-min infusion (a) or a 6-hr infusion (b). (——) Lymph concentration (left scale); (– –) cumulative recovery (right scale).

formulations. These studies were done in sheep after the efferent lymphatic duct of the popliteal lymph node was cannulated. This provides an excellent model to study the pharmacokinetical behavior of rIFN alpha-2a in lymph and blood. It has the added advantage that the biological effects of rIFN alpha-2a on lymphoid tissue can also be determined and correlated to the concentrations measured in lymph and blood. Following a single s.c. or i.d. injection of 2×10^7 U rIFN alpha-2a into the drainage area of the popliteal lymph node, the lymphocyte output fell to below 1% of the pretreatment level and remained depressed for up to 35 hr. After the period of depressed lymphocyte output, a seemingly compensatory surge of cell traffic occurred which lasted 2–3 days (14).

Our data show that following i.d./s.c. administration into the cannulated leg, 59.2 ± 7.2% (mean ± SD; $N = 8$) of the administered rIFN alpha-2a dose is recovered in the efferent popliteal lymph. This result indicates that after i.d./s.c. administration, rIFN alpha-2a (MW 19,000) is absorbed mainly by the lymphatics which drain the application site. Under the conditions of our experiments, lymph containing rIFN alpha-2a is diverted from the animal and does not enter the blood vascular circulation. Thus the relatively low blood plasma levels of rIFN alpha-2a observed after i.d. administration into the cannulated leg (maximum measured concentration, 38 U/ml) compared with the concentration after i.d. administration into the opposite, noncannulated leg (maximum measured concentration, 315 U/ml) are further evidence that after i.d. administration most of the rIFN alpha-2a does not enter the bloodstream directly through blood capillaries but indirectly by way of lymphatics.

This finding can be compared with data published previously by Muranishi and co-workers (15). They evaluated the absorption route (lymph or blood vessels) of macromolecules following rectal administration. They found that small amounts of the administered dose of macromolecules can pass the epithelium of the rectum with the help of enhancers. After this passage they do not readily enter the blood vessels but instead easily pass into the lymphatics. A macromolecule smaller than MW 10,000 may be transported into both fluids at about the same concentrations, while a protein with a MW of approximately 20,000 may enter principally into the lymphatics.

In previous investigations in rabbits Bocci *et al.* studied the absorption characteristics of interferon following s.c. or i.d. administration. They showed that during s.c. injection the coadministration of albumin increased the lymphatic absorption of interferon and delayed its appearance in blood (9,16,17). In contrast to the s.c. route, the interferon distribution after i.d. application was not influenced by the addition of albumin (18). Under our experimental conditions the cumulative recovery of rIFN alpha-2a in lymph was affected neither by the addition of albumin nor by the administration route (s.c. or i.d.). The only effect we observed was a delayed appearance of rIFN alpha-2a in the efferent popliteal lymph when administered in a 13% human albumin solution.

The concentration profiles in lymph and blood plasma following parenteral administration of rIFN alpha-2a revealed that after i.d. administration very high rIFN alpha-2a lymph concentrations can be obtained, while blood plasma levels remain relatively low. The peak concentration of rIFN alpha-2a measured in the efferent popliteal lymph following i.d. administration was 10^5 times higher than after i.v. administration of the same dose. The lymph/blood plasma ratios following i.d. administration[5] ranged between 8000 at 30 min and 2000 at 2 hr, whereas the ratios following i.v. administration ranged between 0.002 at 30 min and 0.14 at 2 hr.

Bocci (3) postulated that a physiological distribution of interferon (lymph/blood plasma ratio, >1) might increase its therapeutic index. Our results demonstrate that this lymph/blood plasma ratio can be achieved easily by i.d. or s.c. administration. It must be considered that this physiological rIFN alpha-2a distribution is achieved only locally. The ratios of the rIFN alpha-2a concentration in the lymph vessels of the opposite site to that in the blood plasma are similar to the ones following i.v. administration and range between 0.01 at 30 min and 1 at 2 hr. However, administration at several different sites could adjust the lymph/blood plasma ratio to a more physiological one over a larger portion of the peripheral immune system.

In intact animals, lymphocytes recirculate continually from the blood to the lymph, and most of this migration occurs in lymph nodes (19). It has been shown in sheep that 30–50 million lymphocytes recirculate each hour through

[5] The lymph/blood plasma ratios following i.d. administration were calculated from the concentrations in the efferent popliteal lymph following i.d. administration into the lymph cannulated leg and the blood plasma levels following i.d. administration into the opposite, noncannulated leg.

1 g lymph node (20) and there is no reason why other animals, including humans, should differ greatly in this regard. Therefore, by continuous delivery of rIFN alpha-2a, or perhaps other protein drugs, to one or more lymph nodes, a significant proportion of the peripheral lymphocyte pool would be exposed periodically to high levels of the drug. By maintaining this special microenvironment, it may become feasible to alter the immunological activity of more widely dispersed lymphoid tissues without the disadvantage of having high levels of drug in the general circulation, when undesirable side effects can follow.

In summary, we have shown that following s.c. or i.d. administration rIFN alpha-2a (MW 19,000) is absorbed mainly by the lymphatics. This results in high rIFN alpha-2a levels in the lymphoid tissue which drains the application site, while blood plasma levels are relatively low. The maximum measured concentrations of rIFN alpha-2a in the efferent popliteal lymph varied by a factor of 10^5 between i.d./s.c. and i.v. administration. The knowledge gained in this investigation may help to improve the mode of administration and therapeutic efficacy of protein drugs whose targets are lymphoid cells.

ACKNOWLEDGMENTS

We thank Misses L. Dudler, E. Infanger, and I. Pracht for technical assistance, Mrs. C. Merten for typing the manuscript, and Dr. G. Cirelli for the design of the injection device. We also wish to acknowledge Drs. C. Surber and P. Haefelfinger for helpful discussions.

REFERENCES

1. J. Drews. *Immunpharmakologie,* Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1986.
2. V. Bocci. *J. Biol. Resp. Modif.* 4:340–352 (1985).
3. V. Bocci. *Immunol. Today.* **6**:7–9 (1985).
4. V. Bocci. *Cancer Drug Deliv.* **1**:337–351 (1984).
5. H. Yoshikawa, K. Takada, S. Muranishi, Y.-I. Satoh, and N. Naruse. *J. Pharmaco-Dyn.* 7:59–62 (1984).
6. H. Yoshikawa, K. Takada, Y.-I. Satoh, N. Naruse, and S. Muranishi. *Pharm. Res.* 2:249–250 (1985).
7. H. Yoshikawa, Y.-I. Satoh, N. Naruse, K. Takada, and S. Muranishi. *J. Pharmaco-Dyn.* 8:206–210 (1985).
8. H. Yoshikawa, K. Takada, Y.-I. Satoh, N. Naruse, and S. Muranishi. *Pharm. Res.* 3:116–117 (1986).
9. V. Bocci, M. Muscettola, G. Grasso, Z. Magyar, A. Naldini, and G. Szabo. *Experientia* **42**:432–433 (1986).
10. S. Pestka. *Arch. Biochem. Biophys.* **221**:1–37 (1983).
11. J. G. Hall and B. Morris. *Q. J. Exp. Physiol.* **47**:360–369 (1962).
12. H. Gallati. *J. Clin. Chem. Clin. Biochem.* **22**:907–914 (1982).
13. P. W. Trown, R. J. Wills, and J. J. Kamm. *Cancer* **57**:1648–1656 (1986).
14. W. Hein and A. Supersaxo. *Immunology* (in press).
15. S. Muranishi, K. Takada, H. Yoshikawa, and M. Murakami. In S. S. Davis, L. Illum, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs,* Plenum Press, New York and London, 1986, pp. 177–189.
16. V. Bocci, M. Muscettola, A. Naldini, E. Bianchi, and G. Segre. *Gen. Pharmacol.* **17**:93–96 (1986).
17. V. Bocci, M. Muscettola, and A. Naldini. *Gen. Pharmacol.* **17**:445–448 (1986).
18. V. Bocci, M. Muscettola, and A. Naldini. *Int. J. Pharm.* **32**:103–110 (1986).
19. B. Morris. In *Handbuch der allgemeinen Pathologie, Vol. 3,* Springer-Verlag, Berlin, 1972, Part 6, pp. 405–456.
20. J. G. Hall and B. Morris. *J. Exp. Med.* **121**:901–910 (1965).