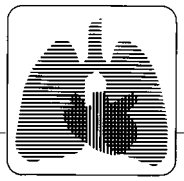

special report

Chicken Soup Inhibits Neutrophil Chemotaxis *In Vitro**

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Chicken soup has long been regarded as a remedy for symptomatic upper respiratory tract infections. As it is likely that the clinical similarity of the diverse infectious processes that can result in "colds" is due to a shared inflammatory response, an effect of chicken soup in mitigating inflammation could account for its attested benefits. To evaluate this, a traditional chicken soup was tested for its ability to inhibit neutrophil migration using the standard Boyden blindwell chemotaxis chamber assay with zymosan-activated serum and fMet-Leu-Phe as chemoattractants. Chicken soup significantly inhibited neutrophil migration and did so in a concentration-dependent manner. The activity was present in a nonparticulate component of the chicken soup. All of the vegetables present in the soup and the chicken individually had inhibitory activity, although only the chicken lacked cytotoxic activity. Interestingly, the complete soup also lacked cytotoxic activity. Commercial soups varied greatly in their inhibitory activity. The present study, therefore, suggests that chicken soup may contain a number of substances with beneficial medicinal activity. A mild anti-inflammatory effect could be one mechanism by which the soup could result in the mitigation of symptomatic upper respiratory tract infections.

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Key words: chicken soup; neutrophil chemotaxis

Abbreviations: fMLP = fMet-Leu-Phe; HBSS = Hank's balanced salt solution; ZAS = zymosan-activated serum

Chicken soup has been regarded as a remedy for centuries. The Egyptian Jewish physician and philosopher Moshe ben Maimon (Maimonides) recommended chicken soup for respiratory tract symptoms in his 12th century treatise, reportedly drawing on classical Greek sources.[1–4] So widely recommended is chicken soup in the Jewish tradition, that it is referred to by a variety of synonyms as Jewish penicillin, bohbymycetin, and bobamycin.[5,6] Chicken soup is, however, also recommended for similar purposes in a variety of other traditions suggesting multiple independent discoveries.[7]

Colds are generally the result of transient infections of the mucosa of the upper respiratory tract with a variety of viruses including, but not limited to, the rhinoviruses.[8–10] While incompletely understood, the viral infection leads to the stimulation of a cytokine cascade.[11,12] It is likely that many, if not most, of the symptoms related to colds are consequent to the inflammatory response thus initiated.[13–15] The activation of common physiologic pathways likely accounts for the marked similarity of symptoms that result from colds. In this regard, colds are associated with the generation of neutrophil chemotactic activities[11–13,15] and with the recruitment of neutrophils to the epithelial surface of the airways.[12,13,16,17] Since neutrophil products are potent secretagogues,[18] this may be one mechanism by which colds commonly lead to cough and sputum from a diverse set of infections.

Chicken soup may have a number of beneficial effects for an individual with a cold. These could

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include actions as diverse as improving hydration and nutritional status[19] and accelerating mucosal clearance.[5] The nature of the direct cytotoxic actions on microorganisms are controversial.[6,20,21] Another potential mechanism for beneficial effects could be an attenuation of the inflammatory response. In order to evaluate that possibility, the ability of chicken soup to inhibit neutrophil chemotaxis in response to standard chemotactic stimuli was evaluated and demonstrated in the current study. These results provide one mechanistic basis in support of the traditional claims made for chicken soup as a remedy.

Materials and Methods

Soup

Traditional chicken soup was prepared according to a family recipe, which will be referred to as "Grandma's soup" (C. Fleischer; personal communication; 1970). This recipe is as follows:

- 1 5- to 6-lb stewing hen or baking chicken;
- 1 package of chicken wings;
- 3 large onions;
- 1 large sweet potato;
- 3 parsnips;
- 2 turnips;
- 11 to 12 large carrots;
- 5 to 6 celery stems;
- 1 bunch of parsley; and
- salt and pepper to taste.

Clean the chicken, put it in a large pot, and cover it with cold water. Bring the water to a boil. Add the chicken wings, onions, sweet potato, parsnips, turnips, and carrots. Boil about 1.5 h. Remove fat from the surface as it accumulates. Add the parsley and celery. Cook the mixture about 45 min longer. Remove the chicken. The chicken is not used further for the soup. (The meat makes excellent chicken parmesan.) Put the vegetables in a food processor until they are chopped fine or pass through a strainer. Both were performed in the present study. Salt and pepper to taste. (Note: this soup freezes well.) Matzoh balls were prepared according to the recipe on the back of the box of matzoh meal (Manischewitz; Jersey City, NJ).

Three separate preparations of soup were made. The completed soup was collected from all three. In addition, in order to determine at which stage the soup acquired activity, 19 samples were collected during the preparation of one batch (Table 1). As the mixture was inhomogeneous, several samples were collected at the same time from different regions of the pot. All samples were frozen in small aliquots and stored (−80°C) until assay.

In order to determine whether particulates accounted for the activity of the soup, attempts were made to obtain a clarified preparation. Soup that was prepared by this method could not be passed through a 0.22-μm filter. In order to remove particulates, 1-mL aliquots were centrifuged (12,000*g* for 15 min) in Eppendorf tubes. This resulted in a visible pellet and a clarified transparent yellow supernatant, which was aspirated for subsequent assay.

To determine which components of the soup contained inhibitor activity, samples of chicken (a leg) and a portion of each of the vegetables were boiled for approximately 1 h. The supernatant broths then were harvested, frozen, and saved for assay.

Table 1—Sample Descriptions

Sample	Cooking Time, min	Description
1	0	Bird in water
2	38	Bird in boiling water
3	51	Immediately after adding vegetables
4	72	Under chicken
5	72	Top of pot near onions
6	72	Top of pot near carrots
7	83	Bottom of pot
8	83	Top of pot
9	91	Matzoh ball preparation, paste
10	183	Matzoh ball preparation, complete
11	183	Top of pot
12	183	Bottom of pot
13	201	Middle of pot
14	417	Aqueous phase
15	417	"Lipid" phase
16	417	Matzoh ball broth
17	451	Mashed vegetables
18	451	Matzoh balls added
19	486	Completed (needs to be seasoned to taste)

For comparison purposes, commercially available soups were obtained from a local supermarket and prepared according to the directions on the packaging. No strict quality control was performed, although each preparation was evaluated by taste and was felt to be satisfactory (if variably so).

Neutrophil Chemotaxis

Peripheral blood was collected from healthy nonsmoking volunteers under a protocol approved by the University of Nebraska Institutional Review Board and by sedimentation through dextran, as described previously.[22] Neutrophils then were rinsed, suspended at 10^6 cells/mL in Hank's balanced salt solution (HBSS), and used as targets for chemotaxis. Chemotaxis was performed by the modified blindwell technique using 48-well multichambers and 3-μm pore size polycarbonate filters (Nucleoprobe; Cabin John, MD), as described previously.[23]

Soup Inhibition of Neutrophil Chemotaxis

In order to determine whether the soup could inhibit chemotaxis, dilutions of soup (1:100) were added to the top and bottom wells of the chemotaxis chamber. Zymosan-activated serum (ZAS)[24] was used as the positive chemoattractant. As a control to determine whether chicken soup had chemotactic activity, chicken soup was added directly to the bottom of the chemotaxis chamber without other chemoattractants.

Concentration Dependence of Chicken Soup Effect

Serial dilutions of chicken soup were added to neutrophils in the upper portion of the chemotaxis chamber, and ZAS (1:4) or fMet-Leu-Phe (fMLP), 10^{-7} mol/L (Sigma; St. Louis, MO), were used as chemoattractants.

Viability

In order to determine whether soup and its components were cytotoxic, neutrophils were prepared as if for chemotaxis and

were suspended in HBSS with a 1:100 dilution of soup, component vegetables, or chicken extracts. After a 30-min incubation at 37°C, cells were collected and viability was assessed by trypan blue dye exclusion visually.

Statistics

For data sets with multiple comparisons, analysis of variance was first used to determine whether any group was significantly different, following which Student's *t* test was used for comparisons that appeared to be different. Data presented are mean ± SEM.

Results

Chicken soup was found to inhibit neutrophil chemotaxis. When the completed soup (without added salt and pepper) was added to neutrophils above the membrane, to the ZAS below the membrane, or to both sides of the chemotaxis membrane, neutrophil migration to ZAS was inhibited. The effect of the chicken soup was much more marked when the diluted chicken soup was added directly to the neutrophils (Fig 1). The diluted chicken soup by itself had a minimal effect in stimulating neutrophil chemotaxis above background in the absence of a chemoattractant (Fig 1).

The inhibitory effect of chicken soup was concentration-dependent and was observed when both ZAS and fMLP were used as chemoattractants (Fig 2). Interestingly, chicken soup added to the neutrophils at dilutions of $> 1{:}20$ caused a slight but significant ($p < 0.05$) increase in neutrophil migration toward media controls (Fig 2). Three preparations of chicken soup inhibited chemotaxis to ZAS similarly (Fig 3). It was not possible to remove the particulate matter from the chicken soup by filtration. In order to determine whether the solid component of the chicken soup might be responsible for the inhibition of chemotaxis, therefore, the chicken soup was clarified by centrifugation at high speed. Although there was some loss of activity, the clarified supernatants of the three chicken soup preparations retained the majority of inhibitory activity (Fig 3).

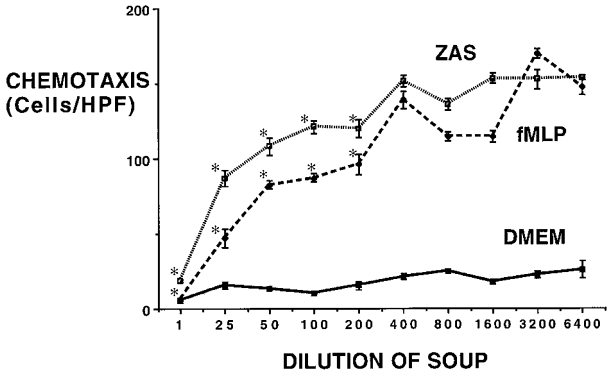

Figure 2. Inhibition of neutrophil chemotaxis and concentration dependence. Chicken soup (preparation No. 1, fraction No. 18) was placed in varying dilutions together with neutrophils in the upper portion of the chemotaxis chamber. ZAS, fMLP, or Dulbecco's modified Eagle's medium (DMEM) was placed in the lower portion of the chemotaxis chamber as a chemoattractant. Chemotaxis then was performed. * = $p < 0.05$ compared to the highest dilution. HPF = high-power field.

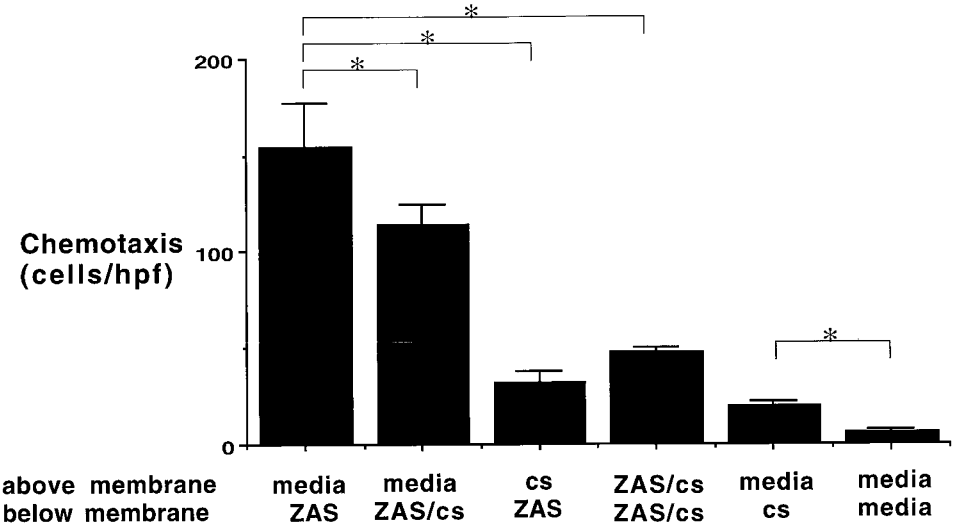

Figure 1. Inhibition of neutrophil chemotaxis by chicken soup. ZAS (dilution, 1:4) and chicken soup (cs) (prep 1, fraction 18 diluted 1:100) were added to the top and bottom of the chemotaxis chamber in various combinations as indicated. Neutrophils were added to the top of the chamber, and neutrophil chemotaxis performed. Vertical axis: migrated neutrophils (cells per high-power field [hpf]). Horizontal axis: condition. * = $p < 0.05$.

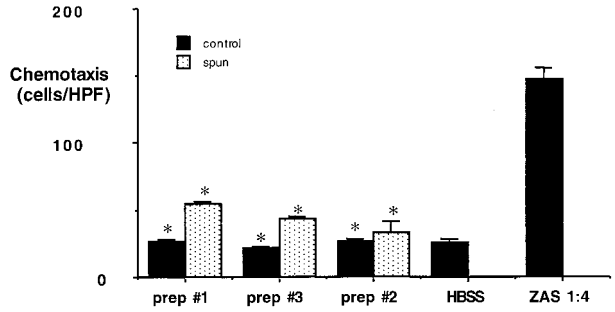

FIGURE 3. Inhibition of chemotaxis by various preparations and the effect of clarification. Three separate preparations of chicken soup were tested either in the completed stage (solid bars) or after clarification by centrifugation (15 min, 12,000*g*, stipple bars). All samples were diluted 1:40 into HBSS and were added together with the neutrophils. ZAS, diluted 1:4, was used as the chemoattractant. HBSS and ZAS alone are shown as negative and positive controls, respectively. Vertical axis: migrated neutrophils. Horizontal axis: conditions. * = $p < 0.05$ compared to ZAS control. See Figure 2 for other abbreviations.

In an attempt to partially determine which components of chicken soup had activity, two experimental approaches were undertaken. First, samples of chicken soup were harvested at various times during the preparation. As the soup preparation was exceedingly inhomogeneous, samples were taken from various parts of the pot at various times. Samples containing the initial stages of the soup with early chicken broth alone were not active (Fig 4). All samples harvested after the addition of the first group of vegetables had inhibitory activity. In the final stages of the preparation, slightly less inhibition of chemotaxis was observed. Second, an analysis of individual soup components was performed by boiling individual components. All ingredients were found to be inhibitory, including the boiled extract of chicken alone (Fig 5, *top*). The effect on the inhibition of the whole soup was not due to effects on neutrophil viability. The viability of neutrophils exposed to each of the complete chicken soup preparations was always $> 95\%$. The viability of the neutrophils incubated in the boiled chicken stock was 98%. The isolated vegetable components, interestingly, demonstrated a slight, but statistically significant, loss of neutrophil viability, as assessed by trypan blue exclusion (Fig 5, *bottom*).

In a modest attempt to determine whether commercially available preparations of chicken soup also inhibited neutrophil chemotaxis, 13 different soups were purchased at a local supermarket and were tested against Grandma's soup (Fig 6). Many of the soups inhibited neutrophil chemotaxis. Five inhibited more potently (at an identical dilution) than did Grandma's traditional soup. Two soups were without activity, and one slightly augmented chemotaxis. Omaha tap water had no activity.

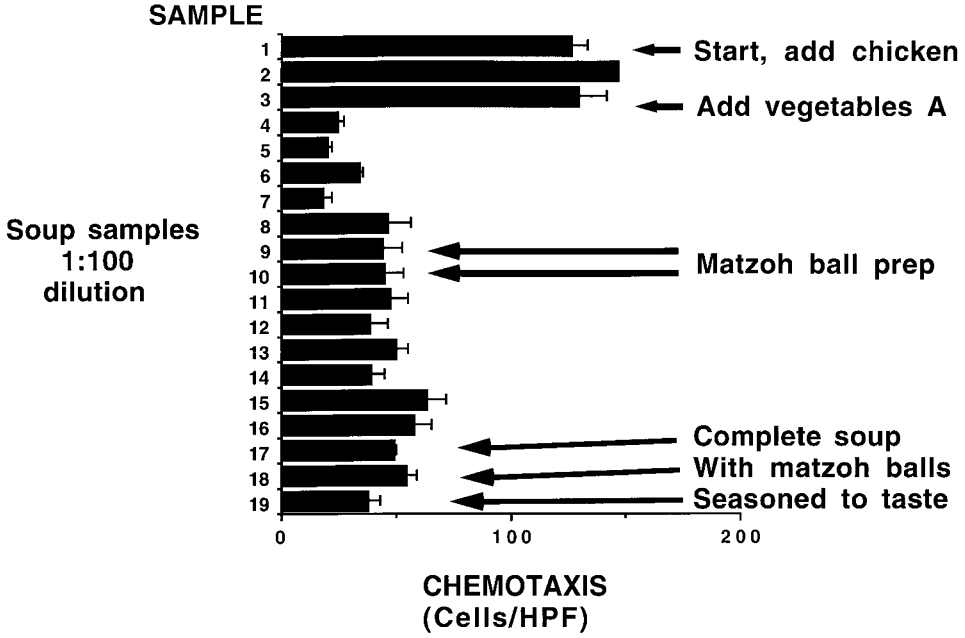

FIGURE 4. Acquisition of chemotactic inhibitory activity during preparation of the soup. Samples were collected at various stages during the preparation of chicken soup. Each aliquot then was diluted 1:100 into HBSS and was added together with neutrophils in the top portion of the chemotaxis chamber. Chemotaxis then was measured using ZAS diluted 1:4 as the chemoattractant. The major stages of the soup preparation are indicated. See Figure 2 for other abbreviations.

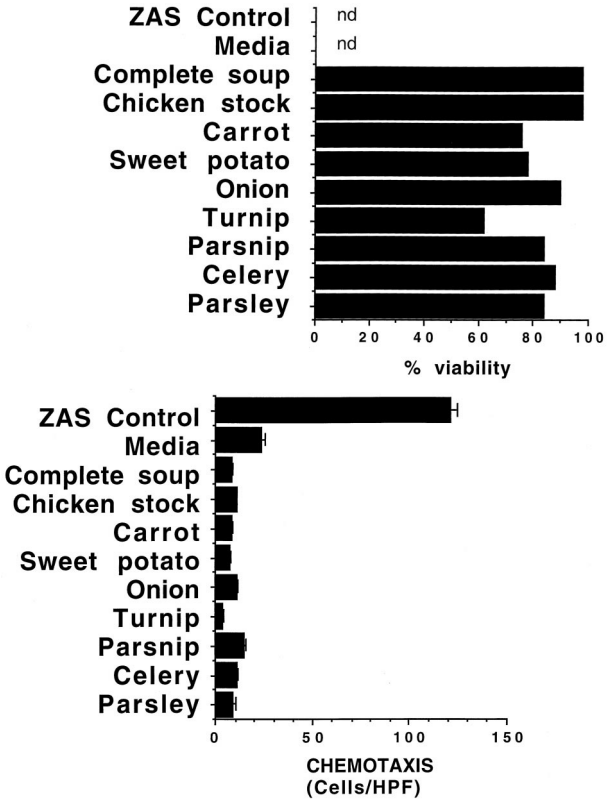

FIGURE 5. The effect of specific vegetable ingredients on the inhibition of chemotaxis and neutrophil viability. *Top*: the component ingredients of chicken soup were boiled in water after which they were sonicated, diluted 1:100 in HBSS, and added to neutrophils in the top portion of a chemotaxis chamber. ZAS diluted 1:4 was used as the chemoattractant. *Bottom*: viability was assessed by trypan blue dye exclusion after incubating the neutrophils in HBSS with the diluted soup components for 30 min. See Figure 2 for other abbreviations.

DISCUSSION

The current study demonstrates that chicken soup inhibits neutrophil migration to standard stimuli as assessed by the modified Boyden blindwell chamber method. The effect appears to be due to an effect on the neutrophils rather than on the chemoattractant, as addition of the soup directly to the neutrophils appears to be most effective. The inhibitory effect was observed clearly at concentrations without cytotoxicity, as determined by trypan blue dye exclusion. Finally, a variety of soup preparations was evaluated and found to be variably, but generally, able to inhibit neutrophil chemotaxis. The current study, therefore, presents evidence that chicken soup might have an anti-inflammatory activity, namely, the inhibition of neutrophil migration.

The identity of the active ingredient or ingredients present in the soup remains unknown. The vegetables that are used to prepare the soup, however, are known to contain a large number of chemical species, many of which have medicinal activities.[25–27] A number of fats and substances with antioxidant activity are also likely to be present. Extracts of each vegetable, as well as of the chicken, all were able to inhibit neutrophil chemotaxis, suggesting that many inhibitory substances may be present. Interestingly, the vegetable extracts also demonstrated some neutrophil cytotoxicity that was not observed either in the completed soup or in the chicken extract. No attempt was made to control for concentration of various extracted components, and the toxicity could be due to a concentration-dependent effect. However, the preparation of the soup is a multistep process, and many complex chemical interactions are taking place. Determining these processes quantitatively and preparing appropriately controlled component extracts will be a challenging problem.

It is interesting, however, that neither the chicken nor the completed soup had cytotoxicity. There are several possibilities in addition to concentration effects that could explain such an effect. The chicken may contain a component that chemically neutralizes vegetable-derived toxins. Alternatively, the fat that is slowly extracted from the chicken and then skimmed from the soup surface could be extracting a lipid-soluble toxin from the preparation. That some interaction takes place during the cooking seems likely as the soup acquires maximal inhibitory activity shortly after adding the first group of vegetables. While still active, inhibitory activity does decrease slightly during the later stages of the preparation. Finally, it also is possible that the chicken could contain a component that directly activates neutrophils and has a protective effect, *eg*, by inducing antioxidants.

The current study assessed a single measure of the inflammatory response, migration of neutrophils by the blindwell assay method. Chicken soup inhibited chemotaxis to two different chemoattractants, ZAS, which generates the active fragment of the fifth component of complement, C5a,[24] and fMLP.[28] *In vivo* inflammatory responses are complex and multifaceted. Whether chicken soup has other activities remains to be determined. It was of interest, however, that while able to inhibit chemoattractant-driven migration, the soup had a slight direct chemotactic activity and may have slightly augmented nondirected migration. These effects, while statistically significant, were small and were not pursued in the current study. However, their presence suggests that chicken soup contains a multitude of moieties with diverse physiologic effects.

The chicken soup recipe used for the majority of these experiments is very highly regarded locally.[29] It does have several unusual features, however. First, it contains several vegetables, *eg*, sweet potato, not found in many chicken soup recipes; in addition, in

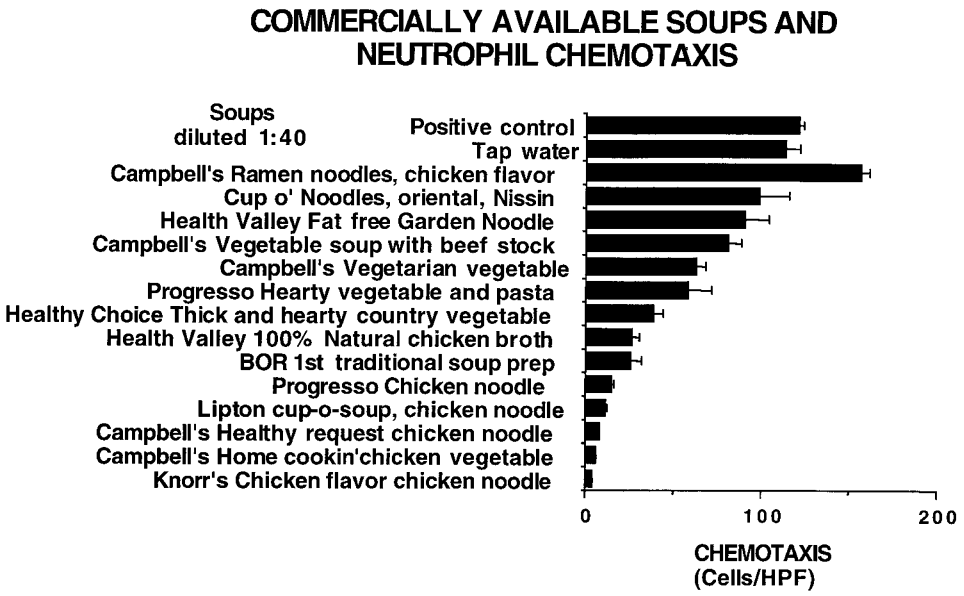

FIGURE 6. The effect of various commercial soups on neutrophil chemotaxis. A number of preparations of soup were purchased at a local supermarket and were prepared according to the instructions on the label. All samples then were diluted 1:40 in HBSS added to neutrophils, and chemotaxis to ZAS (diluted 1:4) was tested. See Figure 2 for other abbreviations.

many recipes, the vegetables are removed from the clear broth prior to serving. After removal, Grandma's soup calls for the vegetables to be pureed and added to the soup. (We understand that this was a modification introduced by Grandma during the Great Depression to ensure that everyone ate the available vegetables.) The soup, as a result, contains a thick suspension of particulates.

Particulates can interact with neutrophils and could, perhaps, interfere with chemotaxis.[30] However, for several reasons, it seems unlikely that particulates account for the majority of the activity. First, Grandma's soup, clarified by centrifugation, retained the majority of inhibitory activity. Second, Grandma's soup preparation was active prior to the addition of the pureed vegetables, the major source of particulates. Finally, inhibitory activity was observed with several other recipes that lack the vegetable particulates. Thus, while the identity of the biologically active materials is unknown, it seems likely they are water soluble or extractable.

Whether the active moieties present in chicken soup achieve sufficient concentration to be active following *in vivo* ingestion is not known. The identity of these moieties is not known, and bioavailability testing was beyond the scope of the current study. The activity is water extractable, however, suggesting that it may be absorbable. The inhibitory effect of chicken soup on neutrophil chemotaxis, moreover, was observed at dilutions as low as 1:200. This is comparable to the dilution of a 350-mL "average" bowl of soup eaten by a 70-kg person. The observations that activities are present in the clarified soup, are active at a dilution that was comparable to that of one bowl diluted into a body volume, and are water extractable are consistent, when taken together, with a potential *in vivo* effect.

Undoubtedly, the *in vivo* effects of chicken soup include more than the effects on neutrophils. The warm liquid, particularly when sipped, can stimulate nasal clearance and may improve upper respiratory tract symptoms.[5] The social setting in which chicken soup is often taken is likely to contribute to a strong placebo effect. Despite the observation that neutrophil chemotactic inhibitors are present in many vegetable extracts, pureed carrots (or other vegetables) are not recommended as a remedy, while chicken soup is. This suggests that whole chicken soup may contain a mixture of active agents that synergize each other in order to achieve their beneficial effects. It is also consistent with the recommendation that the use of chickens of a certain age[1] that are, perhaps, happy[31] is more effective. Such a synergism would not be surprising, as it is certainly true for taste (this observation is from common knowledge and general experience).

Chicken soup is not without hazard. Anaphylaxis,[28] aspiration,[32–34] and severe electrolyte disturbances[36,37] all have been described as a result of chicken soup ingestion. An anti-inflammatory effect could increase the risk for secondary infection. The benefits of chicken soup, however, are widely acclaimed

and have been the subject of several reviews,[1,7] although the anecdotal nature of the clinical evidence supporting a benefit of chicken soup is well recognized.[20,21] These benefits range from alleviation of symptoms of respiratory tract infection,[1–3,5–7] to possibly improving aircraft fuel usage,[37] although the data supporting some of these various claims are meager, and many of these reports lack scientific vigor. The current study was well controlled and used well-established *in vitro* methods to provide limited evidence that chicken soup could have an anti-inflammatory activity. Since many of the symptoms that follow upper respiratory tract viral infections may well be due to the inflammatory response, the current study may have clinical relevance.

Prolonged benefits of chicken soup also have been reported in some settings.[38] It has been suggested that even transient respiratory tract inflammation can cause prolonged worsening of asthma.[39] Should chicken soup reduce respiratory tract inflammation *in vivo*, there may be a prolonged benefit. It is more difficult, however, to relate the results of the present study to some of the other claims made for chicken soup, but the authors have no doubt that such speculations have been made in good taste.

The current study demonstrates a statistically significant inhibition of neutrophil chemotaxis by chicken soup *in vitro*. This was not an *in vivo* clinical trial. Whether clinical benefits would be obtained with the chicken soup used in the current study or not, therefore, remains untested. Many readers of this journal will have had personal experience with the ingestion of chicken soup in the setting of respiratory tract symptoms or other illnesses. Many clinically efficacious therapies have been discovered through careful observation. The present study provides one piece of evidence that chicken soup contains compounds of potential medical value. No doubt, many other traditional remedies do as well. The evaluation of traditional remedies by rigorous modern methods has the potential to expand our therapeutic armamentarium.

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